(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 March 2003 (06.03.2003)

PCT

(10) International Publication Number
WO 03/018632 A2

(51) International Patent Classification[7]: C07K 14/72, 14/59, 16/28, C12N 5/10, 15/12, G01N 33/53, 33/564, A61K 39/00, 38/17

(21) International Application Number: PCT/GB02/03831

(22) International Filing Date: 21 August 2002 (21.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0120649.9	23 August 2001 (23.08.2001)	GB
0215212.2	1 July 2002 (01.07.2002)	GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/018632 A2

(54) Title: EPITOPE REGIONS OF A THYROTROPHIN (TSH) RECEPTOR, USES THEREOF AND ANTIBODIES THERETO

(57) Abstract: The present invention is concerned with epitope regions of a thyrotrophin (TSH) receptor, uses thereof and antibodies thereto.

Epitope Regions of a Thyrotrophin (TSH) Receptor, Uses thereof and Antibodies thereto

The present invention is concerned with epitope regions of a thyrotrophin (TSH) receptor, uses thereof and antibodies thereto.

Thyrotrophin or thyroid stimulating hormone (TSH) is a pituitary hormone which plays a key role in regulating the function of the thyroid. Its release is stimulated by the hormone TRH formed in the hypothalamus and controls the formation and release of the important thyroid hormones thyroxine (T4) and tri-iodothyronine (T3). On the basis of a feedback mechanism, the thyroid hormone content of the serum controls the release of TSH. The formation of T3 and T4 by the thyroid cells is stimulated by TSH by a procedure in which the TSH released by the pituitary binds to the TSH receptor of the thyroid cell membrane.

In certain pathological conditions, various types of autoantibodies against this TSH receptor can also be formed. Depending on the type of these autoantibodies, either inhibition of the formation and release of T3 and T4 may occur at the TSH receptor owing to the shielding of the TSH molecules, or, on the other hand, these thyroid hormones may be released in an uncontrolled manner because the anti-TSH receptor autoantibodies mimic the action of the TSH and stimulate the synthesis and release of thyroid hormones.

Autoimmune thyroid disease (AITD) is the most common autoimmune disease affecting different populations worldwide. A proportion of patients with AITD, principally those with Graves' disease, have autoantibodies to the TSH receptor substantially as hereinbefore described. The autoantibodies bind to the TSH receptor and usually mimic the actions of TSH, stimulating the thyroid gland to produce high levels of thyroid hormones. These autoantibodies are described as having stimulating activity. In some patients, autoantibodies bind to the TSH receptor but do not stimulate thyroid hormone production and are described as having blocking activity [J Sanders, Y Oda, S-A Roberts, M Maruyama, J Furmaniak, B Rees Smith "Understanding the thyrotrophin receptor

function-structure relationship." Baillière's Clinical Endocrinology and Metabolism. Ed. T F Davies 1997 11(3): 451-479. Pub. Baillière Tindall, London].

Measurements of TSH receptor autoantibodies are important in the diagnosis and management of AITD, particularly Graves' disease. Currently three types of assays are used to measure TSH receptor autoantibodies:

(a) competitive binding assays which measure the ability of TSH receptor autoantibodies to inhibit the binding of TSH to preparations of TSH receptor;

(b) bioassays which measure the ability of TSH receptor autoantibodies to stimulate cells expressing the TSH receptor in culture; and

(c) immunoprecipitation of TSH receptor preparations with TSH receptor autoantibodies.

Measurement of TSH receptor autoantibodies using such assays are described in references J Sanders, Y Oda, S-A Roberts, M Maruyama, J Furmaniak, B Rees Smith "Understanding the thyrotrophin receptor function-structure relationship." Baillière's Clinical Endocrinology and Metabolism. Ed. T F Davies 1997 11(3): 451-479. Pub. Baillière Tindall, London, and J Sanders, Y Oda, S Roberts, A Kiddie, T Richards, J Bolton, V McGrath, S Walters, D Jaskólski, J Furmaniak, B Rees Smith "The interaction of TSH receptor autoantibodies with ^{125}I-labelled TSH receptor." Journal of Clinical Endocrinology and Metabolism 1999 84(10):3797-3802.

There are, however, a number of limitations associated with the use of the above described currently available assays for measuring TSH receptor autoantibodies. The competitive assays of type (a) which are available in different formats are generally sensitive, relatively easy to perform and adaptable for routine use. However, competitive radioreceptor assays known to date for detecting TSH receptor autoantibodies have

fundamental disadvantages of a practical nature which can be ascribed to the fact that the binding ability of TSH receptor preparations generally react very sensitively to changes in the receptor or in a biomolecule bound by it. The binding of biomolecules which are peptides or protein in nature, for example hormones or autoantibodies, to receptors is as a rule very complicated in nature, and the specific binding between receptor and biomolecule is very much more sensitive to structural alterations, in particular of the receptor, than is the case with a usual antigen/antibody binding pair which is the basis of most immunoassays in which receptors are involved. Attempts to immobilise and / or to label the TSH receptor have as a rule led to structural alterations which have greatly impaired the functionality of the receptor.

As far as bioassays of the type mentioned in (b) are concerned, these tend to be expensive, time-consuming, require highly skilled staff and are essentially unsuitable for routine use.

With respect to the direct immunoprecipitation assays of type (c), currently available such immunoprecipitation assays do not in practice have the required sensitivity for TSH receptor autoantibody detection.

The present invention alleviates the problems hitherto associated with the prior art detection of TSH receptor autoantibodies. More particularly, the present invention provides diagnostic methods and kits for screening for TSH receptor autoantibodies, with improved sensitivity compared to prior art diagnostic methods and kits, and which, if desired, allow the use of one or more competitive binding partners or competitors for a TSH receptor in competitive assays of the type described above. In particular the present invention is concerned with the use of one or more identified epitope regions of TSH receptor in diagnostic methods and kits for screening for TSH receptor autoantibodies.

There is provided by the present invention, therefore, for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising part or all of the primary structural conformation (that is a continuous sequence of amino acid residues) of one or more TSH receptor epitopes with

which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments);

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) with said polypeptide sequence, so as to enable said diagnosis or therapy.

More particularly, there is provided by the present invention for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide

sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments);

wherein autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) with said polypeptide sequence, so as to enable said diagnosis or therapy.

Alternatively, there is provided by the present invention for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments);

wherein lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) with said polypeptide sequence, so as to enable said diagnosis or therapy.

The present invention further provides for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments);

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) with said polypeptide sequence, so as to enable said diagnosis or therapy.

More particularly, the present invention further provides for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments);

wherein autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) with said polypeptide sequence, so as to enable said diagnosis or therapy.

The present invention further provides for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments);

wherein lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) with said polypeptide sequence, so as to enable said diagnosis or therapy.

More preferably, it is generally preferred that such diagnostic or therapeutic use employs a polypeptide sequence or sequences comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor;

(in particular said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers

277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or the primary structural conformation of amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 247 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments).

In particular, it is generally preferred according to the present invention that such diagnostic or therapeutic use employs amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

In particular, it is generally preferred according to the present invention that such diagnostic or therapeutic use employs amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

In particular, it is generally preferred according to the present invention that such diagnostic or therapeutic use employs amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

A particularly preferred such diagnostic or therapeutic use according to the present invention, comprises for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more

variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments;

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) with said polypeptide sequences, so as to enable said diagnosis or therapy.

More particularly, such diagnostic or therapeutic use may comprise:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments;

wherein autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) with said polypeptide sequences, so as to enable said diagnosis or therapy.

Alternatively, such diagnostic or therapeutic use may comprise:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or

consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments;

wherein lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) with said polypeptide sequences, so as to enable said diagnosis or therapy.

A particularly preferred diagnostic or therapeutic use according to the present invention, comprises for use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the

primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments;

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) with said polypeptide sequences, so as to enable said diagnosis or therapy.

More particularly, such diagnostic or therapeutic use may comprise:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of

amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments;

wherein autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) with said polypeptide sequences, so as to enable said diagnosis or therapy.

Alternatively, such diagnostic or therapeutic use may comprise:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments

of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments;

wherein lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) with said polypeptide sequences, so as to enable said diagnosis or therapy.

It may also be further preferred that the above mentioned diagnostic or therapeutic use employing:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments;

further employs:

(iii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor, or variants, analogs or derivatives of such fragments.

More particularly, such preferred diagnostic or therapeutic use employs:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments;

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow

interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments; and

(iii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or variants, analogs or derivatives of such fragments.

As will be appreciated from the accompanying Figures, the above mentioned amino acid sequences can be of human, porcine, bovine, canine, feline, mouse, rat or ovine origin, and the specific amino acid sequences in each of the above mentioned species are hereinafter described in greater detail with reference to Figures 1, 3, 5, and 7.

There also provided by the present invention one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of

such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments).

More particularly, there is provided by the present invention one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such

fragments).

Alternatively, there is provided by the present invention one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments).

The present invention further provides one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;

amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments).

More particularly, the present invention further provides one or more TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), said one ore more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such

fragments; or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments).

The present invention further provides one or more TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), said TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments).

More preferably, it is generally preferred that one or more TSH receptor epitopes comprise one or more of the following, or one or more variants, analogs, derivatives or fragments

thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; or amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 247 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments).

A particularly preferred TSH receptor epitope according to the present invention comprises, consists of or consists essentially of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes).

A particularly preferred TSH receptor epitope according to the present invention comprises, consists of or consists essentially of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes).

A particularly preferred TSH receptor epitope according to the present invention comprises, consists of or consists essentially of amino acid numbers 247 to 260 of a TSH

receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes).

There is also provided by the present invention a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments), with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), with the exception of a full length TSH receptor.

More particularly, there is provided by the present invention a polypeptide with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) and which comprises, consists of or consists essentially of part or all of the primary structural conformation (that is a continuous sequence of amino acid residues) of one or more epitopes of a TSH receptor with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments), with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), with the exception of a full length TSH receptor.

Alternatively, there is provided by the present invention a polypeptide with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) and which comprises, consists of or consists essentially of part or all of the primary structural conformation (that is a continuous sequence of amino acid residues) of one or more

epitopes of a TSH receptor with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

(in particular amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments), with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), with the exception of a full length TSH receptor.

The present invention further provides a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or

derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments), with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), with the exception of a full length TSH receptor.

More particularly, the present invention further provides a polypeptide with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments), with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), with the exception of a full length TSH receptor.

The present invention further provides a polypeptide with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;

amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, (in particular amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and / or amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments), with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), with the exception of a full length TSH receptor.

More preferably, it is generally preferred that a polypeptide according to the present invention can comprise part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and as such comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes):

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;

amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor.

Preferably a polypeptide according to the present invention comprises, consists of or consists essentially of, amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

Preferably a polypeptide according to the present invention comprises, consists of or consists essentially of, amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

Preferably a polypeptide according to the present invention comprises, consists of or consists essentially of, amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

It is also preferred according to the present invention that there is provided a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in

response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

with the exception of a full length TSH receptor.

More particularly, there is provided by the present invention a polypeptide with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies produced in response to a TSH

receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies);

with the exception of a full length TSH receptor.

Alternatively, there is provided by the present invention a polypeptide with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes);

with the exception of a full length TSH receptor.

The present invention further provides a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under

conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises part or all of the primary structural conformation of epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

with the exception of a full length TSH receptor.

More particularly, the present invention further provides a polypeptide with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with

which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments, with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments, with which autoantibodies produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies);

with the exception of a full length TSH receptor.

The present invention further provides a polypeptide with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a

TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments, with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes); and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments, with which lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such lymphocytes);

with the exception of a full length TSH receptor.

It is also preferred according to the present invention that there is provided a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises part or all of the primary structural conformation of TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow

interaction of a TSH receptor with such autoantibodies or lymphocytes);

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes); and

(iii) the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

with the exception of a full length TSH receptor.

More particularly, the present invention further provides a polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes) and which comprises part or all of the primary structural conformation of epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes), which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of

Figure 5, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

(iii) the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact (suitably under conditions that allow interaction of a TSH receptor with such autoantibodies or lymphocytes);

with the exception of a full length TSH receptor.

As will be appreciated from the accompanying Figures, such amino acid sequences can be of human, porcine, bovine, canine, feline, mouse, rat or ovine origin, and the specific amino acid sequences in each of the above mentioned species are hereinafter described in greater detail with reference to Figures 1, 3, 5, and 7. Suitably, in the case where polypeptides according to the second aspect of the present invention comprise amino acid sequences corresponding to part or all of the primary structural conformation of more than

one epitope of a TSH receptor, the respective amino acid sequences corresponding to part or all of the primary structural conformation of respective epitopes may be separated by linker amino acid sequences so as to preferably provide the respective amino acid sequences in a conformation, arrangement or sequence that resembles or substantially resembles a conformation, arrangement or sequence of amino acids as present in an active site of a TSH receptor, and / or can be effective in providing the above referred to respective amino acid sequences of a TSH receptor in a conformation, arrangement or sequence optimal for interaction with autoantibodies and / or lymphocytes as described herein.

Preferred polypeptide sequences and polypeptides according to the present invention comprise, consist of, or consist essentially of, the specifically referred to amino acid numbered sequences of a TSH receptor as respectively shown in any of accompanying Figures 1, 3, 5 or 7. As indicated above, however, the present invention also covers "variants", "analogs", "derivatives" and "fragments" of specific amino acid sequences described herein and the terms "variants", "analogs", "derivatives" and "fragments" as used herein when referring to polypeptide sequences and polypeptides according to the present invention (such as polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures) can be characterised as polypeptide sequences and polypeptides which retain essentially the same biological function or activity (in terms of autoantibody and / or lymphocyte interaction as described herein) as polypeptide sequences and polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures. Suitably, variants, analogs, derivatives and fragments, or variants, analogs or derivatives of the fragments as described herein can have a primary structural conformation of amino acids as seen in the accompanying Figures in which several or a few (such as 5 to 10, 1 to 5 or 1 to 3) amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions are deletions which do not alter or substantially alter the biological activity or function of polypeptides according to the present invention as specifically described above. Conservative substitutions can be preferred as hereinafter

described in greater detail.

More particularly, variants, analogs or derivatives of polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures may be:

(i) ones in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue); or

(ii) ones in which one or more of the amino acid resides includes a substituent group; or

(iii) ones which further comprise additional amino acids that can be effective in providing the above referred to amino acid numbers of a TSH receptor that are present in a polypeptide of the present invention in a conformation, arrangement or sequence that resembles or substantially resembles a conformation, arrangement or sequence of amino acids as present in an active site of a TSH receptor, and / or can be effective in providing the above referred to amino acid numbers of a TSH receptor that are present in a polypeptide of the present invention in a conformation, arrangement or sequence optimal for interaction with autoantibodies and / or lymphocytes as described herein.

Such variants, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Typically, variants, analogs or derivatives can be those that vary from a reference (such as polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures) by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a

polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids A, V, L and I; among the hydroxyl residues S and T; among the acidic residues D and E; among the amide residues N and Q; among the basic residues K and R; and among the aromatic residues F and Y.

It may be preferred that variants, analogs or derivatives as provided by the present invention are ones which further comprise additional amino acids that can be effective in providing the above referred to amino acid numbers of a TSH receptor that are present in a polypeptide of the present invention in a conformation, arrangement or sequence that resembles or substantially resembles a conformation, arrangement or sequence of amino acids as present in an active site of a TSH receptor, and / or can be effective in providing the above referred to amino acid numbers of a TSH receptor that are present in a polypeptide of the present invention in a conformation, arrangement or sequence optimal for interaction with autoantibodies and / or lymphocytes as described herein.

More particularly, the term "fragment" as used herein denotes a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of a polypeptide having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures, and variants or derivatives thereof and such fragments may be "free standing", i.e. not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. As will be appreciated, fragments according to the present invention comprise or contain the primary structural conformation of amino acids present in one or more epitopes of a TSH receptor as described herein so as to be capable of interaction with autoantibodies and / or lymphocytes as described herein.

Polypeptides of the present invention, therefore, include polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures as well as polypeptides (namely variants, analogs and derivatives as referred to above) having at least 70% identity to polypeptides having a primary

structural conformation of specified amino acids as described herein with reference to the accompanying Figures, preferably at least 80% identity to the polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures, and more preferably at least 90% identity to polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures and still more preferably at least 95% identity to polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures and also includes fragments of such polypeptides substantially as referred to above.

A polypeptide according to the present invention is suitably obtained by, or is obtainable by, expression of a polynucleotide according to the present invention substantially as hereinafter described. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesisers employing techniques which are well known in the art. A polypeptide according to the present invention so obtained can be advantageous in being free from association with other eukaryotic polypeptides or contaminants which might otherwise be associated therewith in its natural environment.

Polypeptides according to the present invention substantially as herein described can be expressed in various systems generating recombinant proteins. For example, for expression in *E coli*, cDNA coding for the appropriate polypeptides according to the present invention can be cloned into a vector, such as pET22, pMEX8, pGEX2T or pQE-81L His or an equivalent. In the case of expression in yeast (for example *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*), vectors such as pYES2, pESP2 or pYES2/CT or an equivalent, can be employed. AcMNPV (Bac-N-Blue) vector or an equivalent can be used for expression in insect cells and pRC/CMV, pcDNA3.1 vectors or an equivalent can be used for expression in mammalian cells, such as Chinese Hamster Ovary (CHO) cells. A polypeptide according to the present invention can be expressed as a discrete protein, or as a fusion protein linked to, for example, glutathione S transferase (GST) or poly histidine linker. For a discrete protein, affinity column chromatography purification using a mouse monoclonal antibody to the relevant part of a polypeptide according to the

present invention coupled to a Sepharose particle can be used. If a polypeptide according to the present invention is fused to GST, glutathione Sepharose chromatography purification can be used to isolate the fusion protein. Specific proteases can be used to separate GST from a polypeptide according to the present invention and a second round of glutathione Sepharose chromatography can be used to separate GST from a polypeptide according to the present invention. In the case of peptides linked to poly histidine linker, the purification can be carried out using immobilised metal affinity chromatography.

The present invention further provides a process of preparing a polypeptide substantially as hereinbefore described, which process comprises:

(i) providing a host cell substantially as hereinbefore described;

(ii) growing the host cell; and

(iii) recovering a polypeptide according to the present invention therefrom.

Recovery of a polypeptide according to the present invention can typically employ conventional isolation and purification techniques, such as chromatographic separations or immunological separations, known to one of ordinary skill in the art.

In accordance with a further aspect of the present invention, there is provided a polynucleotide comprising:

(i) a nucleotide sequence encoding a polypeptide substantially as hereinbefore described;

(ii) a nucleotide sequence encoding a polypeptide substantially as hereinbefore described, which polypeptide comprises an amino acid sequence or sequences of specified amino acid numbers of a TSH receptor which is or are defined by reference to any of Figures 1, 3, 5 and 7;

(iii) a nucleotide sequence encoding a polypeptide of (ii), which nucleotide sequence comprises nucleotide bases encoding the above mentioned specified amino acid numbers of a TSH receptor which are defined by reference to any of Figures 1, 3, 5, and 7, and which nucleotide bases are defined by reference to any of Figures 2, 4, 6 and 8;

(iv) a nucleotide sequence differing from the sequence of (iii) in codon sequence due to the degeneracy of the genetic code;

(v) a nucleotide sequence comprising an allelic variation of the sequence of (iii);

(vi) a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v); or

(vii) a nucleotide sequence which hybridizes under stringent conditions to any of the sequences of (i), (ii), (iii), (iv), (v) or (vi).

The nucleotide bases of a polynucleotide according to the present invention, encoding the above mentioned epitope regions of a polypeptide according to the present invention, can be summarised as follows.

Amino Acid Numbers	Nucleotide Numbers
22-91	64-273
32-41	94-123
36-42	106-126
246-260	736-780
247-260	739-780
260-363	778-1089
277-296	829-888

380-418	1138-1254
381-385	1141-1155

Polynucleotides of the present invention may be in the form of DNA, including, for instance, cDNA, synthetic DNA and genomic DNA appropriately obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. A preferred embodiment of the present invention preferably comprises cDNA or synthetic DNA.

The coding sequence which encodes a polypeptide according to the present invention may be identical to the coding sequence of a polynucleotide as referred to above in (iii) and defined by reference to any of Figures 2, 4, 6 and 8. It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes a polypeptide according to the present invention.

The present invention further relates to variants of the herein above described polynucleotides which encode for polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures, variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of the fragments and substantially as hereinbefore described in greater detail. A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques.

Among the variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions, again substantially as hereinbefore described.

Variant polynucleotides according to the present invention are suitably at least 70%

identical over their entire length to a polynucleotide encoding polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures, and polynucleotides which are complementary to, or hybridise to, such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding a polypeptides having a primary structural conformation of specified amino acids as described herein with reference to the accompanying Figures and polynucleotides which are complementary to, or hybridise to, such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% identity are especially preferred. Furthermore, those with at least 97% identity are highly preferred among those with at least 95% identity, and among these those with at least 98% identity and at least 99% identity are particularly highly preferred, with at least 99% identity being the more preferred.

Substantially as hereinbefore described the present invention further relates to polynucleotides that hybridise to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridise under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridisation will occur only if there is at least 95% and preferably at least 97% complementary identity between the sequences.

The present invention also relates to vectors, which comprise a polynucleotide or polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

The present invention, therefore, further provides a biologically functional vector system which carries a polynucleotide substantially as hereinbefore described and which is capable of introducing the polynucleotide into the genome of a host organism.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention and the present invention further provides a host cell which is transformed or transfected with a polynucleotide, or one or more polynucleotides, or a vector system, each substantially as herein described. The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques.

According to a particularly preferred embodiment of the present invention, there is also provided a method of screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing either (i) said sample of body fluid from said subject or (ii) lymphocytes isolated from said sample;

(b) contacting said sample or isolated lymphocytes with a polypeptide according to the present invention substantially as hereinbefore described (suitably under conditions that allow interaction of a TSH receptor with autoantibodies or lymphocytes produced in response to a TSH receptor) so as to permit said polypeptide to interact with autoantibodies, or lymphocytes, produced in response to a TSH receptor, and present in, or isolated from, said sample; and

(c) monitoring the degree, or effect, of interaction of said polypeptide with either said autoantibodies, or said lymphocytes, produced in response to a TSH receptor and present in, or isolated from, said sample, thereby providing an indication of the presence of said autoantibodies, or said lymphocytes, in said sample, or isolated from said sample.

Substantially as described above, a method according to the present invention is suitable

for screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject. A method according to the present invention can, however, be particularly adapted for use in screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject substantially as hereinafter described in greater detail.

There is in particular provided by the present invention, therefore, a method of screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with a polypeptide according to the present invention substantially as hereinbefore described (suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said polypeptide to interact with autoantibodies produced in response to a TSH receptor and present in said sample; and

(c) monitoring the degree of interaction of said polypeptide with said autoantibodies produced in response to a TSH receptor and present in said sample, thereby providing an indication of the presence of said autoantibodies in said sample.

A method according to the present invention may typically employ a control, such as a sample of body fluid from a normal subject, in other words a subject known to be without autoimmune disease associated with an immune reaction to a TSH receptor.

A method of screening for autoantibodies to a TSH receptor according to the present

invention may comprise directly monitoring interaction of (i) autoantibodies to a TSH receptor present in the sample of body fluid from the subject and (ii) a polypeptide, as provided by the present invention substantially as hereinbefore described, typically by employing non-competitive sandwich type assay techniques known in the art.

Typically, in a method according to the present invention employing non-competitive techniques, monitoring of the degree of interaction of (i) autoantibodies to a TSH receptor present in the sample and (ii) a polypeptide according to the present invention substantially as hereinbefore described, can comprise providing labelling means either to a polypeptide according to the present invention substantially as hereinbefore described, or to a binding partner for autoantibodies to a TSH receptor, either of which technique would enable monitoring of the above described interaction. For example, a method according to the present invention may comprise directly or indirectly labelling a polypeptide according to the present invention substantially as hereinbefore described; contacting the thus labelled polypeptide with a sample of body fluid being screened for TSH receptor autoantibodies so as to provide a mixture thereof; and adding to the mixture a binding partner for autoantibodies to a TSH receptor (such as an anti -IgG reagent) present in the sample of body fluid, so as to cause precipitation of any complexes of labelled polypeptide and TSH receptor autoantibodies present in the mixture. Alternatively, it may be preferred that a method according to the present invention further comprises adding a labelled binding partner for TSH receptor autoantibodies (such as a labelled anti- IgG reagent, for example protein A or anti-human IgG, or labelled full length TSH receptor or an epitope thereof) to a mixture obtained by contacting (i) a polypeptide according to the present invention substantially as hereinbefore described immobilised to a support and (ii) a sample of body fluid being screened for autoantibodies to a TSH receptor.

It may alternatively be preferred that a method of screening for autoantibodies to a TSH receptor in the sample of body fluid according to the present invention, utilises the principles employed in known competitive assays. For example, a method according to the present invention may employ at least one competitor capable of competing with

autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described.

Typically, a competitor as employed in a competitive assay method according to the present invention may comprise one or more antibodies, which may be natural or partly or wholly synthetically produced. A competitor as employed in the present invention may alternatively comprise any other protein (for example TSH) having a binding domain or region which is capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described. Preferably, however, a competitor as employed in the present invention comprises a monoclonal, recombinant or polyclonal antibody (especially a monoclonal antibody), capable of competing with TSH receptor autoantibodies in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described.

Typically, therefore, a competitive assay method according to the present invention may further comprise providing at least one competitor, such as a monoclonal or polyclonal antibody, whereby in step (b) of a method as herein described a polypeptide according to the present invention substantially as hereinbefore described can interact with either a competitor, such as a monoclonal or polyclonal antibody, or autoantibodies to a TSH receptor present in said sample.

Typically monitoring in a competitive assay method according to the present invention comprises comparing:

(i) interaction of a polypeptide according to the present invention substantially as hereinbefore described and one or more competitors substantially as hereinbefore described (typically a monoclonal or polyclonal antibody), in the absence of said sample of body fluid being screened (that is a suspected disease sample), optionally in the presence of a sample of body fluid from a normal subject, typically a subject known

to be without autoimmune disease associated with an immune reaction to a TSH receptor; with

(ii) interaction of a polypeptide according to the present invention substantially as hereinbefore described and one or more competitors substantially as hereinbefore described (typically a monoclonal or polyclonal antibody), in the presence of said sample of body fluid being screened.

Typically, the comparison involves observing a decrease in interaction of a polypeptide according to the present invention substantially as hereinbefore described and the competitor in (ii) compared to (i) so as to provide an indication of the presence of autoantibodies to a TSH receptor in said sample. Typically, the decrease in interaction can be observed by directly or indirectly labelling the competitor and monitoring any change in the interaction of the thus labelled competitor with a polypeptide according to the present invention substantially as hereinbefore described in the absence and in the presence of a sample of body fluid being screened for autoantibodies to a TSH receptor. Suitably a polypeptide according to the present invention substantially as hereinbefore described may be immobilised to facilitate the above mentioned monitoring.

Alternatively, there is also provided by the present invention a method of screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor (typically a recombinantly

obtained full length TSH receptor), and

(ii) at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described,

(suitably under conditions that allow interaction of a TSH receptor with autoantibodies to a TSH receptor), so as to permit said full length TSH receptor to interact with either autoantibodies to a TSH receptor present in said sample, or said competitor; and

(c) monitoring the interaction of said full length TSH receptor with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

The full length TSH receptor can typically be of human, porcine, bovine, canine, feline, mouse, rat or ovine origin and more preferably a recombinantly obtained full length TSH receptor. A competitor for use in such an assay typically comprises a monoclonal or polyclonal antibody (preferably monoclonal) substantially as hereinbefore described.

Suitably a detectable label that can be employed in a method according to the present invention can be selected from the group consisting of enzymic labels, isotopic labels, chemiluminescent labels, fluorescent labels, dyes and the like

In the case where an isotopic label (such as ^{125}I, ^{14}C, ^{3}H or ^{35}S) is employed, monitoring may therefore comprise measuring radioactivity dependent on binding of a polypeptide according to the present invention substantially as hereinbefore described. Radioactivity is generally measured using a gamma counter, or liquid scintillation counter.

In the case of a method of screening for lymphocytes according to the present invention,

it is generally preferred that lymphocytes are initially isolated from a sample of body fluid from a subject using techniques well known to one of ordinary skill in the art, followed by contact with a polypeptide according to the present invention so as to stimulate the proliferation of the isolated lymphocytes. Monitoring of the effect of interaction of a polypeptide according to the present invention and such proliferating lymphocytes, typically employs means known in the art for monitoring such proliferation of lymphocytes.

According to a further particularly preferred embodiment of the present invention, there is provided a kit for screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a polypeptide according to the present invention substantially as hereinbefore described;

(b) means for contacting either (i) a sample of body fluid obtained from said subject, or (ii) lymphocytes isolated from a sample of body fluid obtained from said subject, with said polypeptide according to the present invention substantially as hereinbefore described (suitably under conditions that allow interaction of a TSH receptor with autoantibodies or lymphocytes produced in response to a TSH receptor) so as to permit said polypeptide to interact with autoantibodies, or lymphocytes, produced in response to a TSH receptor, and present in, or isolated from, said sample; and

(c) means for monitoring the degree, or effect, of interaction of said polypeptide with either said autoantibodies, or said lymphocytes, produced in response to a TSH receptor and present in, or isolated from, said sample, thereby providing an indication of the presence of said autoantibodies, or lymphocytes, in said sample or isolated from said sample.

Substantially as described above, a kit according to the present invention is suitable for screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject. A kit according to the present invention can, however, be particularly adapted for use in screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject substantially as hereinafter described in greater detail.

There is in particular provided by the present invention, therefore, a kit for screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a polypeptide according to the present invention substantially as hereinbefore described;

(b) means for contacting a sample of body fluid obtained from said subject with said polypeptide according to the present invention substantially as hereinbefore described (suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said polypeptide to interact with autoantibodies produced in response to a TSH receptor and present in said sample; and

(c) means for monitoring the degree of interaction of said polypeptide with said autoantibodies produced in response to a TSH receptor and present in said sample, thereby providing an indication of the presence of said autoantibodies in said sample.

A kit according to the present invention may typically further comprise control means, such as means for providing a sample of body fluid from a normal subject, in other words a subject known to be without autoimmune disease associated with an immune reaction

to a TSH receptor.

A kit for screening for autoantibodies to a TSH receptor according to the present invention may comprise means for directly monitoring interaction of (i) autoantibodies to a TSH receptor present in the sample of body fluid from the subject and (ii) a polypeptide, as provided by the present invention substantially as hereinbefore described, typically comprising non-competitive sandwich type assay means known in the art.

Typically, in a kit according to the present invention comprising non-competitive assay means, means are provided for monitoring the degree of interaction of (i) autoantibodies to a TSH receptor present in the sample and (ii) a polypeptide according to the present invention substantially as hereinbefore described, and can comprise labelling means provided either to a polypeptide according to the present invention substantially as hereinbefore described, or to a binding partner for autoantibodies to a TSH receptor, either of which would enable monitoring of the above described interaction. For example, a kit according to the present invention may comprise means for directly or indirectly labelling a polypeptide according to the present invention substantially as hereinbefore described; means for contacting the thus labelled polypeptide with a sample of body fluid being screened for a TSH receptor autoantibodies so as to provide a mixture thereof; a binding partner for autoantibodies to a TSH receptor (such as an anti -IgG reagent) present in the sample of body fluid; and means for adding the binding partner to the mixture so as to cause precipitation of any complexes of labelled polypeptide and TSH receptor autoantibodies present in the mixture. Alternatively, it may be preferred that a kit according to the present invention further comprises a labelled binding partner for TSH receptor autoantibodies (such as a labelled anti- IgG reagent, for example protein A or anti-human IgG, or labelled full length a TSH receptor or an epitope thereof) and means for adding the labelled binding partner to a mixture obtained by contacting (i) a polypeptide according to the present invention substantially as hereinbefore described immobilised to a support and (ii) a sample of body fluid being screened for autoantibodies to a TSH receptor.

It may alternatively be preferred that a kit for screening for autoantibodies to a TSH receptor in the sample of body fluid according to the present invention, comprises known competitive assay means. For example, a kit according to the present invention may further comprise at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described.

Typically, a competitor as employed in a competitive assay kit according to the present invention may comprise one or more antibodies, which may be natural or partly or wholly synthetically produced. A competitor as employed in the present invention may alternatively comprise any other protein having a binding domain or region which is capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described. Preferably, however, a competitor as employed in the present invention comprises a monoclonal or polyclonal antibody (especially a monoclonal antibody), capable of competing with TSH receptor autoantibodies in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described.

Typically, therefore, a competitive assay kit according to the present invention may further comprise at least one competitor, such as a monoclonal or polyclonal antibody, whereby a polypeptide according to the present invention substantially as hereinbefore described can interact with either a competitor, such as a monoclonal or polyclonal antibody, or autoantibodies to a TSH receptor present in a sample of body fluid being screened.

Typically monitoring means in a competitive assay kit according to the present invention comprise means for comparing:

(i) interaction of a polypeptide according to the present invention substantially as hereinbefore described and one or more competitors substantially as hereinbefore described (typically a monoclonal or polyclonal antibody), in the absence of said sample of body fluid being

screened (that is a suspected disease sample), optionally in the presence of a sample of body fluid from a normal subject, typically a subject known to be without autoimmune disease associated with an immune reaction to a TSH receptor ; with

(ii) interaction of a polypeptide according to the present invention substantially as hereinbefore described and one or more competitors substantially as hereinbefore described (typically a monoclonal or polyclonal antibody), in the presence of said sample of body fluid being screened.

Typically, the comparison involves observing a decrease in interaction of a polypeptide according to the present invention substantially as hereinbefore described and the competitor in (ii) compared to (i) so as to provide an indication of the presence of autoantibodies to a TSH receptor in said sample. Typically, the decrease in interaction can be observed by directly or indirectly labelling the competitor and monitoring any change in the interaction of the thus labelled competitor with a polypeptide according to the present invention substantially as hereinbefore described in the absence and in the presence of a sample of body fluid being screened for autoantibodies to a TSH receptor. Suitably a polypeptide according to the present invention substantially as hereinbefore described may be immobilised to facilitate the above mentioned monitoring.

Alternatively, there is also provided by the present invention a kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor (typically a recombinantly obtained full length TSH receptor);

(b) at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to the present invention substantially as hereinbefore described,

(c) means for contacting said sample of body fluid from said subject, said full length TSH receptor and said competitor (suitably under conditions that allow interaction of a TSH receptor with autoantibodies to a TSH receptor), so as to permit said full length TSH receptor to interact with either autoantibodies to a TSH receptor present in said sample, or said competitor; and

(d) means for monitoring the interaction of said full length TSH receptor with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

The full length TSH receptor can typically be of human, porcine, bovine, canine, feline, mouse, rat or ovine origin and more preferably a recombinantly obtained full length TSH receptor. A competitor for use in such an assay kit typically comprises a monoclonal or polyclonal antibody (preferably monoclonal) substantially as hereinbefore described.

Suitably a detectable label that can be employed in a kit according to the present invention can be selected from the group consisting of enzymic labels, isotopic labels, chemiluminescent labels, fluorescent labels, dyes and the like.

In the case where an isotopic label (such as ^{125}I, ^{14}C, ^{3}H or ^{35}S) is employed, monitoring means may therefore comprise means for measuring radioactivity dependent on binding of a polypeptide according to the present invention substantially as hereinbefore described. Radioactivity is generally measured using a gamma counter, or liquid scintillation counter.

In the case of a kit for screening for lymphocytes according to the present invention, it is generally preferred that means are provided for initially isolating lymphocytes from a

sample of body fluid from a subject, using techniques well known to one of ordinary skill in the art, and means are also provided for contacting a polypeptide according to the present invention with such isolated lymphocytes so as to stimulate proliferation of the latter by the former. Means (again known to one of ordinary skill in the art) for monitoring the effect of interaction of a polypeptide according to the present invention and such proliferating lymphocytes, are also provided in such a kit according to the present invention.

It will be appreciated from the foregoing description that the present invention provides assay methods and kits for detecting autoantibodies (in particular) or lymphocytes produced in response to a TSH receptor in a sample of body fluid substantially as hereinbefore described. The detection of such autoantibodies and / or lymphocytes produced in response to a TSH receptor in the sample of body fluid (or at least the level of such autoantibodies and / or lymphocytes in the sample) is indicative of the presence of autoimmune disease associated with an immune reaction to a TSH receptor in the subject from which the sample was obtained and can, therefore, enable the diagnosis of the likely onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor.

There is, therefore, further provided by the present invention a method of diagnosing the likely onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in a subject (in particular a human) suspected of suffering from, susceptible to, having or recovering from, autoimmune disease associated with an immune reaction to a TSH receptor, the method comprising detecting autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid from the subject substantially as hereinbefore described, and whereby the detected autoantibodies and / or lymphocytes can provide a diagnosis of the likely onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in the subject.

There is still further provided by the present invention a method of delaying or preventing the onset of autoimmune disease associated with an immune reaction to a TSH receptor

in an animal subject (in particular a human subject) suspected of suffering from, susceptible to or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, which method comprises initially detecting autoantibodies or lymphocytes indicative of the onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in a sample of body fluid obtained from the subject substantially as hereinbefore described, thereby providing a diagnosis of the likely onset of autoimmune disease associated with an immune reaction to a TSH receptor in the subject, and thereafter therapeutically treating the subject so as to delay the onset and / or prevent autoimmune disease associated with an immune reaction to a TSH receptor.

A polypeptide according to the present invention substantially as hereinbefore described is particularly suitable for use in the therapeutic treatment of autoimmune disease associated with an immune reaction to a TSH receptor. For example, tolerance can be achieved by administering a polypeptide according to the present invention substantially as hereinbefore described to a subject (in particular a human subject) suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor.

There is, therefore, further provided by the present invention a pharmaceutical composition comprising a polypeptide according to the present invention substantially as hereinbefore described, together with a pharmaceutically acceptable carrier, diluent or excipient therefor, wherein the polypeptide can interact with autoantibodies and / or lymphocytes produced in response to a TSH receptor.

The present invention further provides a polypeptide according to the present invention substantially as hereinbefore described for use in the manufacture of a medicament for the treatment of Graves' disease.

Compositions or medicaments according to the present invention should contain a therapeutic or prophylactic amount of at least one polypeptide according to the present invention in a pharmaceutically-acceptable carrier. The pharmaceutical carrier can be any

compatible, non-toxic substance suitable for delivery of the polypeptides to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be used as the carrier. Pharmaceutically-acceptable adjuvants, buffering agents, dispersing agents and the like, may also be incorporated into the pharmaceutical compositions. Such compositions can contain a single polypeptide or may contain two or more polypeptides according to the present invention.

It may be desirable to couple a polypeptide according to the present invention to immunoglobulins, e.g. IgG, or to lymphoid cells from the patient being treated in order to promote tolerance. Such an approach is described in Bradley-Mullen, *Activation of Distinct Subsets of T Suppressor Cells with Type III Pneumococcal Polysaccharide Coupled to Syngeneic Spleen Cells*, in: IMMUNOLOGICAL TOLERANCE TO SELF AND NON-SELF, Buttisto et al., eds., Annals N.Y. Acad. Sci. Vol. 392, pp 156-166, 1982. Alternatively, the polypeptides may be modified to maintain or enhance binding to the MHC while reducing or eliminating binding to the associated T-cell receptor. In this way, the modified polypeptides may compete with natural a TSH receptor to inhibit helper T-cell activation and thus inhibit the immune response. In all cases, care should be taken that administration of the pharmaceutical compositions of the present invention ameliorate but do not potentiate the autoimmune response.

Pharmaceutical compositions according to the present invention are useful for parenteral administration. Preferably, the compositions will be administered parenterally, i.e. subcutaneously, intramuscularly, or intravenously. Thus, the invention provides compositions for parenteral administration to a patient, where the compositions comprise a solution or dispersion of the polypeptides in an acceptable carrier, as described above. The concentration of the polypeptides in the pharmaceutical composition can vary widely, i.e. from less than about 0.1% by weight, usually being at least about 1% by weight to as much as 20% by weight or more. Typical pharmaceutical compositions for intramuscular injection would be made up to contain, for example, 1 ml of sterile buffered water and 1 to 100 μg of a purified polypeptide of the present invention. A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile Ringer's

solution and 100 to 500 mg of a purified polypeptide of the present invention. Actual methods for preparing parenterally administrable compositions are well known in the art and described in more detail in various sources, including, for example, *Remington's Pharmaceutical Science*, 15th Edition, Mack Publishing Company, Easton, Pa. (1980).

In addition to using a polypeptide according to the present invention directly in pharmaceutical compositions, it is also possible to use a polypeptide according to the present invention to enhance tolerance to a TSH receptor in a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, employing the following principles. More particularly, peripheral blood lymphocytes can be collected from the subject in a conventional manner and stimulated by exposure to a polypeptide according to the present invention, as defined above. Usually, other mitogens and growth enhancers will be present, e.g., phytohemagglutinin, interleukin 2, and the like. Proliferating T-helper cells may be isolated and cloned, also under the stimulation of a polypeptide according to the present invention. Clones which continue to proliferate may then be used to prepare therapeutic compositions for the subject. The cloned T-cells may be attenuated, e.g. by exposure to radiation, and administered to the subject in order to induce tolerance. Alternatively, the T-cell receptor or portions thereof may be isolated by conventional protein purification methods from the cloned T-cells and administered to the individual. Such immunotherapy methods are described generally in Sinha et al. (1990) *Science* 248:1380-1388.

In some cases, after a T-helper cell has been cloned as described above, it may be possible to develop therapeutic peptides from the T-cell receptor, where the peptides would be beneficial for treating a patient population suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor. In such cases, the T-cell receptor gene may be isolated and cloned by conventional techniques and peptides based on the receptor produced by recombinant techniques as described above. The recombinantly-produced peptides may then be incorporated in pharmaceutical compositions as described above.

There is also provided by the present invention a method of cloning lymphocytes produced in response to a TSH receptor, which method comprises:

providing a source of lymphocytes;

contacting the lymphocytes with a polypeptide according to the present invention substantially as hereinbefore described, so as to effect proliferation of said lymphocytes; and

isolating and cloning the proliferating lymphocytes.

The present invention also provides the use of cloned lymphocytes prepared as above, in the therapeutic treatment of autoimmune disease associated with an immune reaction to a TSH receptor. There is provided, therefore, a pharmaceutical composition comprising cloned lymphocytes prepared as above, together with a pharmaceutically acceptable carrier, diluent or excipient therefor and the use of such cloned lymphocytes in the manufacture of a medicament for the treatment of autoimmune disease associated with an immune reaction to a TSH receptor, in particular Graves' disease.

There is also provided by the present invention one or more therapeutic agents identified as providing a therapeutic effect by interaction with amino acids comprising part or all of the primary conformation of amino acids of one or more epitopes of a TSH receptor substantially as hereinbefore described, and the present invention further provides one or more therapeutic agents for use in therapeutically interacting with amino acids comprising part or all of the primary conformation of amino acids of one or more epitopes of a TSH receptor substantially as hereinbefore described and as such for use in the therapeutic treatment of an autoimmune disease associated with an immune reaction to a TSH receptor.

There is, therefore, still further provided by the present invention a method of treating autoimmune disease associated with an immune reaction to a TSH receptor in a subject,

which method comprises initially detecting autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from the subject substantially as hereinbefore described, thereby providing a diagnosis of autoimmune disease in the subject, and administering to the subject a therapeutically effective amount of at least one therapeutic agent effective in the treatment of such autoimmune disease, such as a polypeptide according to the present invention substantially as hereinbefore described.

The present invention also provides a method of treating autoimmune disease associated with an immune reaction to a TSH receptor in a subject (in particular a human subject), which method comprises administering to the subject a therapeutically effective amount of a therapeutic agent identified as providing a therapeutic effect by interaction with amino acids comprising part or all of the primary conformation of amino acids of one or more epitopes of a TSH receptor substantially as hereinbefore described

The amount of therapeutic agent administered will depend on the specific autoimmune disease state being treated, possibly the age of the patient and will ultimately be at the discretion of an attendant physician.

There is still further provided by the present invention, in combination, a kit substantially as hereinbefore described, together with a therapeutically effective amount of at least one therapeutic agent effective in the treatment of autoimmune disease associated with an immune reaction to a TSH receptor substantially as hereinbefore described.

Substantially as hereinbefore described, the sample of body fluid being screened by the present invention will typically comprise blood samples or other fluid blood fractions, such as in particular serum samples or plasma samples, but the sample may in principle be another biological fluid, such as saliva or urine or solubilised tissue extracts, or may be obtained by needle biopsy.

There is still further provided by the present invention a binding partner for a TSH

receptor, such as an antibody to a TSH receptor, or a fragment of an antibody to a TSH receptor, which binding partner can interact with one or more epitopes to a TSH receptor substantially as hereinbefore described, in particular amino acid numbers 277 to 296 of a TSH receptor. Suitably, antibodies provided by the present invention can be monoclonal (preferred), recombinant or polyclonal. Typically an antibody, such as a monoclonal antibody, as provided by the present invention is in substantially purified form.

More specifically, a monoclonal antibody as provided by the present invention can comprise any of monoclonal antibodies 3C7, 2B4, 8E2, 18C5, 4D7, 16E5, 17D2, 3B3 and 14D3 or active fragments thereof, as described in the Examples and further illustrated by the accompanying Figures. Antibodies such as 2B4, 8E2, 18C5, 4D7, 16E5, 17D2, 3B3 and 14D3, or active fragments thereof, as described in the Examples preferably have a high affinity for a TSH receptor, such as at least about $10^8 molar^{-1}$. There is, therefore, further provided by the present invention a monoclonal antibody having an affinity of at least about $10^8 molar^{-1}$ for one or more epitopes of a TSH receptor and which epitope is provided by any one of the following amino acid sequences of a TSH receptor:

amino acids 22 to 91 of a TSH receptor; or
amino acids 246 to 260 of a TSH receptor;

or more particularly, consists essentially of any one of the following amino acid sequences of a TSH receptor:

amino acids 36 to 42 of a TSH receptor; or
amino acids 247 to 260 of a TSH receptor.

There is also provided by the present invention a monoclonal antibody having an affinity of at least about $10^8 molar^{-1}$ for one or more epitopes of a TSH receptor and which epitope is provided by any one of the following amino acid sequences of a TSH receptor:

amino acids 32 to 41 of a TSH receptor; or
amino acids 277 to 296 of a TSH receptor.

According to a particularly preferred embodiment of the present invention there is provided a binding partner for a TSH receptor, which binding partner is capable of binding to a TSH receptor so as to stimulate the TSH receptor, which binding partner does not comprise TSH or naturally produced autoantibodies to the TSH receptor.

Preferably the binding partner comprises an antibody, in particular a monoclonal or recombinant (preferably monoclonal) antibody, capable of binding to a TSH receptor so as to stimulate the TSH receptor. Examples of monoclonal antibodies disclosed herein which stimulate a TSH receptor in this way include 4D7, 16E5, 17D2 and 14D3.

In a preferred case the present invention provides a binding partner for a TSH receptor, which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which comprises:

an antibody V_H domain selected from the group consisting of:

V_H domains as shown in any one of Figures 10, 14, 18, 22, 42, 46 or 50, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 10, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 14, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 18, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 22, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 42, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 46, and a V_H domain comprising one or more V_H CDRs with an amino acid sequence

corresponding to a V_H CDR as shown in Figure 50; and / or

an antibody V_L domain selected from the group consisting of:

V_L domains as shown in any one of Figures 12, 16, 20, 24, 44, 48 or 52, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 12, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 16, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 20, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 24, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 44, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 48, and a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 52.

It may be preferred according to the present invention that a binding partner substantially as hereinbefore described comprises an antibody V_H domain substantially as hereinbefore described paired with an antibody V_L domain substantially as hereinbefore described to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor, although as discussed further an antibody V_H domain, or an antibody V_L domain, may be independently used to bind a TSH receptor. It will be appreciated, therefore, that a binding partner substantially as hereinbefore described can comprise an antibody V_H domain substantially as hereinbefore described in the absence of an antibody V_L domain. It will also be appreciated, therefore, that a binding partner substantially as hereinbefore described can comprise an antibody V_L domain substantially as hereinbefore described in the absence of an antibody V_H domain. Alternatively, a binding partner substantially as hereinbefore described can comprise an antibody V_H domain paired with an antibody V_L domain substantially as hereinbefore described to provide an antibody binding site

comprising both V_H and V_L domains for a TSH receptor.

Preferred embodiments according to the present invention can thus include a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 10 paired with an antibody V_L domain as shown in Figure 12 to provide an antibody binding site, comprising both these V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 14 paired with an antibody V_L domain as shown in Figure 16 to provide an antibody binding site, comprising both these V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 18 paired with an antibody V_L domain as shown in Figure 20 to provide an antibody binding site comprising both these V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 22 paired with an antibody V_L domain as shown in Figure 24 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 42 paired with an antibody V_L domain as shown in Figure 44 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 46 paired with an antibody V_L domain as shown in Figure 48 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or a binding partner substantially as hereinbefore described comprising an antibody V_H domain as shown in Figure 50 paired with an antibody V_L domain as shown in Figure 52 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor.

It is further envisaged according to the present invention that V_H domains substantially as hereinbefore described may be paired with V_L domains other than those specifically described herein. It is also further envisaged according to the present invention that V_L domains substantially as hereinbefore described may be paired with V_H domains other than those specifically described herein.

According to an alternative embodiment of the present invention there is provided a binding partner substantially as hereinbefore described for a TSH receptor, which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which can comprise:

an antibody V_H domain comprising:

a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 10, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 14, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 18, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 22, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 42, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 46, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 50; and / or

an antibody V_L domain comprising:

a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 12, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 16, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 20, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 24, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 44, or a V_L domain comprising one or more V_L CDRs with an

amino acid sequence corresponding to a V_L CDR as shown in Figure 48, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 52.

One or more CDRs as referred to above may be taken from the hereinbefore described V_H and V_L domains and incorporated into a suitable framework. For example, the amino acid sequence of one or more CDRs substantially as hereinbefore described may be incorporated into framework regions of antibodies differing from those specifically disclosed herein, such antibodies thereby incorporating the one or more CDRs and being capable of binding to the TSH receptor, preferably to stimulate the TSH receptor substantially as hereinbefore described. Alternatively, a binding partner according to the present invention may comprise a polypeptide capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described and comprising the primary structural conformation of amino acids as represented by one or more CDRs as specifically described herein, optionally together with further amino acids, which further amino acids may enhance the binding affinity of one or more CDRs as described herein for a TSH receptor or may have substantially no role in affecting the binding properties of the polypeptide for a TSH receptor.

Preferably a binding partner according to the present invention includes an antibody. The term "antibody" as used herein describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide having a binding domain which is, or is substantially homologous to, an antibody binding domain. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses and fragments which comprise an antigen binding domain such as Fab, scFv or the like.

In particular, fragments of antibodies specifically as herein described form an important aspect of the present invention. In this way, where a binding partner according to the present invention comprises an antibody substantially as hereinbefore described, the antibody may comprise any of the following fragments: (i) the Fab fragment consisting of V_L, V_H, C_L and C_H1 domains; (ii) the Fd fragment consisting of the V_H and C_H1

domains; (iii) the Fv fragment consisting of the V_L and V_H domains; (iv) the dAb fragment which consists of a V_H domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments; and (vii) single chain Fv molecules (scFv), wherein a V_H domain and a V_L domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site.

Alternatively, in the case where a binding partner according to the present invention comprises an antibody, the antibody may comprise a whole antibody, whereby the antibody includes variable and constant regions, which variable and constant regions can be further illustrated for the antibodies provided by the present invention by reference to any of Figures 9 to 24, or 41 to 52.

The present invention, also encompasses variants, analogs and derivatives of the specific binding partners, antibodies, V_H domains, V_L domains, CDRs and polypeptides disclosed herein, which variants, analogs and derivatives retain the ability to bind to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described. The terms variants, analogs and derivatives are substantially herein before further described in greater detail with respect to polypeptides according to the present invention and what is meant by these terms as hereinbefore described applies also to variants, analogs and derivatives of the specific binding partners according to the present invention.

The present invention also provides a further binding partner capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described, and which further binding partner can compete for binding to the TSH receptor with any specific binding partner disclosed herein, which further binding partner does not comprise TSH or autoantibodies to a TSH receptor. In particular this further binding partner may comprise a further antibody having a binding site for an epitope region of a TSH receptor suitably as hereinbefore described, which further antibody is capable of binding to the TSH receptor so as to stimulate the TSH receptor substantially as hereinbefore described and can compete for binding to the TSH receptor with any specific binding partner disclosed herein.

There is also provided by the present invention a polynucleotide comprising:

(i) a nucleotide sequence as shown in any of Figures 25 to 40, or 53 to 64; or parts of such sequences as shown in Figures 26, 28, 30, 32, 34 ,36, 38, 40, 54, 56, 58, 60, 62, or 64, encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR as shown in any of Figures 10, 12, 14, 16, 18, 20, 22, 24, 42, 44, 46, 48, 50 or 52;

(ii) a nucleotide sequence encoding a binding partner substantially as hereinbefore described, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR of a binding partner substantially as hereinbefore described;

(iii) a nucleotide sequence encoding a binding partner having a primary structural conformation of amino acids as shown in any of Figures 9 to 24 or 41 to 52, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR as shown in any of Figures 10, 12, 14, 16, 18, 20, 22, 24, 42, 44, 46, 48, 50 or 52;

(iv) a nucleotide sequence differing from any sequence of (i) in codon sequence due to the degeneracy of the genetic code.

(v) a nucleotide sequence comprising an allelic variation of any sequence of (i);

(vi) a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v), and in particular a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v) and encoding a Fab fragment, a Fd fragment, a Fv fragment, a dAb fragment, an isolated CDR region, F(ab')2 fragments or a scFv fragment, of a binding partner substantially as hereinbefore described;

(vii) a nucleotide sequence differing from the any sequence of (i) due to mutation, deletion or substitution of a nucleotide base and encoding a binding partner substantially as hereinbefore described, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR of a binding partner substantially as hereinbefore described.

Variant polynucleotides according to the present invention are suitably at least 70% identical over their entire length to any polynucleotide sequence of (i), most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to any polynucleotide sequence of (i), polynucleotides at least 90% identical over their entire length to any polynucleotide sequence of (i) are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% identity are especially preferred. What is meant by variants of specific polynucleotide sequences described herein is hereinbefore described in greater detail.

The present invention further provides a biologically functional vector system which carries a polynucleotide substantially as hereinbefore described and which is capable of introducing the polynucleotide into the genome of a host organism.

The present invention also relates to host cells which are transformed with polynucleotides of the invention and the production of binding partners of the invention by recombinant techniques. Host cells can be genetically engineered to incorporate polynucleotides and express binding partners of the present invention.

A binding partner substantially as hereinbefore described may have diagnostic and therapeutic applications, and may advantageously interact or bind with one or more epitope regions of a TSH receptor substantially as hereinbefore described.

Accordingly, a binding partner substantially as hereinbefore described can be employed in screening methods for detecting autoantibodies substantially as hereinbefore described and also in diagnostic methods substantially as hereinbefore described. In this way,

binding partners according to the present invention can be employed in place of competitors hitherto described for use in screening methods for detecting autoantibodies substantially as hereinbefore described and also in diagnostic methods substantially as hereinbefore described. Similarly, binding partners according to the present invention can be employed in place of competitors hitherto described for use in kits for use in detecting autoantibodies substantially as hereinbefore described.

The present invention also provides a method of screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor, and

(ii) one or more binding partners substantially as hereinbefore described;

(suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding partners; and

(c) monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said

sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

Preferably, a method according to the present invention as referred to above, further comprises providing labelling means for the one or more binding partners, suitable labelling means being substantially as hereinbefore described.

The present invention also provides a method of screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor, and

(ii) one or more binding members for a TSH receptor;

(suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding members; and

(c) monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said

sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample;

wherein said one or more binding members are directly or indirectly immobilised to a surface either prior to, or after step (b).

Typically the one or more binding members comprise one or more binding partners according to the present invention substantially as hereinbefore described. Suitably, labelling means are provided for the TSH receptor, the one or more epitopes thereof or the polypeptide.

The present invention also provides a kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor;

(b) one or more binding partners substantially as hereinbefore described;

(c) means for contacting said sample of body fluid from said subject, said TSH receptor, said one or more epitopes thereof or said polypeptide, and said one or more binding partners, (suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding partners; and

(d) means for monitoring the interaction of said TSH receptor, said one or

more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

Suitably, a kit as referred to above further comprises labelling means for the one or more binding partners, suitable labelling means being substantially as hereinbefore described.

The present invention also provides a kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor;

(b) one or more binding members for a TSH receptor;

(c) means for contacting said sample of body fluid from said subject, said TSH receptor, said one or more epitopes thereof or said polypeptide, and said one or more binding members, (suitably under conditions that allow interaction of a TSH receptor with autoantibodies produced in response to a TSH receptor) so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding members;

(d) means for directly or indirectly immobilising said one or more binding members to a surface, either before or after contacting said one or more binding members with said sample of body fluid from said subject and said TSH receptor, said one or more epitopes thereof or said polypeptide; and

(e) means for monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

Typically the one or more binding members comprise one or more binding partners according to the present invention substantially as hereinbefore described. Suitably, labelling means are provided for the TSH receptor, the one or more epitopes thereof or the polypeptide.

Suitably a method or kit as referred to above can employ a polypeptide or epitope according to the present invention substantially as hereinbefore described.

Substantially as hereinbefore described, in the presence of autoantibodies to the TSH receptor, binding of the TSH receptor to the immobilised binding member or binding partner will be decreased. Such a method and kit for screening for autoantibodies to a TSH receptor can be advantageous in alleviating problems that can be associated with TSH receptor when immobilised to a surface.

A binding partner substantially as hereinbefore described can also be usefully employed in therapy. There is, therefore, further provided by the present invention methods of treatment comprising administration of a specific binding partner substantially as hereinbefore described, pharmaceutical compositions comprising a specific binding partner substantially as hereinbefore described (togther with one or more pharmaceutically acceptable carriers, diluents or excipients therefor), and use of a specific binding partner substantially as hereinbefore described in the manufacture of a medicament or composition, in particular a medicament or composition for use in stimulating thyroid tissue, and / or tissue containing a TSH receptor. In particular, a specific binding partner according to the present invention can be employed in oncology, and in particular for use in the diagnosis, management and treatment of thyroid cancer.

Pharmaceutical compositions according to the present invention include those suitable for oral, parenteral and topical administration, although the most suitable route will generally depend upon the condition of a patient and the specific disease being treated. The precise amount of a binding partner substantially as hereinbefore described to be administered to a patient will be the responsibility of an attendant physician, although the dose employed will depend upon a number of factors, including the age and sex of the patient, the specific disease being treated and the route of administration substantially as described above.

There is further provided by the present invention a method of stimulating thyroid tissue, and / or tissue containing a TSH receptor, which method comprises administering to a patient in need of such stimulation a diagnostically or therapeutically effective amount of a binding partner substantially as hereinbefore described.

The present invention also provides in combination, a binding partner substantially as hereinbefore described, together with one or more further agents capable of stimulating thyroid tissue, and / or tissue containing a TSH receptor, for simultaneous, separate or sequential use in stimulating thyroid tissue, and / or tissue containing a TSH receptor. Preferably the one or more further agents comprise recombinant human TSH and / or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments. Alternatively, the one or more further agents can act independently of binding to the TSH receptor.

The following illustrative explanations are provided to facilitate understanding of certain terms used herein. The explanations are provided as a convenience and are not limitative of the invention

BINDING PARTNER, or BINDING MEMBER, FOR A TSH RECEPTOR, describes a molecule having a binding specificity for a TSH receptor. A binding partner or binding member as described herein may be naturally derived or wholly or partially synthetically produced. Such a binding partner or binding member has a domain or region which specifically binds to and is therefore complementary to one or more epitope regions of a

TSH receptor.

C DOMAIN denotes a region of relatively constant amino acid sequence in antibody molecules.

CDR denotes complementarity determining regions which are present on both heavy and light chains of antibody molecules and represent regions of most sequence variability. CDRs represent approximately 15 to 20% of variable domains and represent antigen binding sites of an antibody.

FR denotes framework regions and represent the remainder of the variable light domains and variable heavy domains not present in CDRs.

HC denotes part of a heavy chain of an antibody molecule comprising the heavy chain variable domain and the first domain of an IgG constant region.

HOST CELL is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

IDENTITY, as known in the art, is the relationship between two or more polypeptide sequences, or two or more polynucleotide sequences, as determined by comparing the sequences.

LC denotes a light chain of an antibody molecule.

STIMULATION OF A TSH RECEPTOR by a binding partner or binding member as described herein denotes the ability of the binding partner or binding member to bind to a TSH receptor and to thereby effect, for example, production of cyclic AMP as a result of such binding to the TSH receptor. Such stimulation is analogous to the responses seen on binding of TSH, or TSH receptor autoantibodies, to a TSH receptor and in this way a binding partner or binding member as described herein mimics the effect of TSH, or TSH

receptor autoantibody, binding to a TSH receptor.

V DOMAIN denotes a region of highly variable amino acid sequence in antibody molecules.

V_HDOMAIN denotes variable regions or domains in heavy chains of antibody molecules.

V_LDOMAIN denotes variable regions or domains in light chains of antibody molecules.

The present invention will now be illustrated by the following Figures and Examples, which do not limit the scope of the invention in any way.

Figure 1 lists amino acids 1 to 200 of (in the following order) human, porcine, bovine, feline, canine, mouse, rat and ovine TSH receptors.

Figure 2 lists nucleotide bases 1 to 300 coding for regions of (in the following order) feline, bovine, canine, mouse, porcine, rat, ovine and human TSH receptors.

Figure 3 lists amino acids 200 to 300 of (in the following order) human, porcine, bovine, feline, canine, mouse, rat and ovine TSH receptors.

Figure 4 lists nucleotide bases 700 to 899 coding for regions of (in the following order) feline, bovine, canine, mouse, porcine, rat, ovine and human TSH receptors.

Figure 5 lists amino acids 250 to 449 of (in the following order) human, porcine, bovine, feline, canine, mouse, rat and ovine TSH receptors.

Figure 6 lists nucleotide bases 750 to 1100 coding for regions of (in the following order) feline, bovine, canine, mouse, porcine, rat, ovine and human TSH receptors.

Figure 7 lists amino acids 350 to 500 of (in the following order) human, porcine, bovine,

feline, canine, mouse, rat and ovine TSH receptors.

Figure 8 lists nucleotide bases 1100 to 1299 coding for regions of (in the following order) feline, bovine, canine, mouse, porcine, rat, ovine and human TSH receptors.

Figure 9 lists amino acids of the heavy chain (HC) of 4D7.

Figure 10 lists amino acids of the heavy chain (HC) of 4D7, showing the variable region or domain (namely amino acid numbers 10 to 115), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 104) and the constant region or domain (namely amino acid numbers 116 to 200).

Figure 11 lists amino acids of the light chain (LC) of 4D7.

Figure 12 lists amino acids of the light chain (LC) of 4D7, showing the variable region or domain (namely amino acid numbers 9 to 111), the CDRs (namely CDR1 amino acid numbers 24 to 38, CDRII amino acid numbers 54 to 60 and CDRIII amino acid numbers 93 to 101) and the constant region or domain (namely amino acids numbers 112 to 211).

Figure 13 lists amino acids of the heavy chain (HC) of 16E5.

Figure 14 lists amino acids of the heavy chain (HC) of 16E5, showing the variable region or domain (namely amino acid numbers 9 to 120), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 109) and the constant region or domain (namely amino acid numbers 121 to 205).

Figure 15 lists amino acids of the light chain (LC) of 16E5.

Figure 16 lists amino acids of the light chain (LC) of 16E5, showing the variable region or domain (namely amino acid numbers 9 to 107), the CDRs (namely CDR1 amino acid numbers 24 to 34, CDRII amino acid numbers 50 to 56 and CDRIII amino acid numbers

89 to 97) and the constant region or domain (namely amino acids numbers 108 to 207).

Figure 17 lists amino acids of the heavy chain (HC) of 17D2.

Figure 18 lists amino acids of the heavy chain (HC) of 17D2, showing the variable region or domain (namely amino acid numbers 9 to 120), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 109) and the constant region or domain (namely amino acid numbers 121 to 205).

Figure 19 lists amino acids of the light chain (LC) of 17D2.

Figure 20 lists amino acids of the light chain (LC) of 17D2, showing the variable region or domain (namely amino acid numbers 9 to 107), the CDRs (namely CDR1 amino acid numbers 24 to 34, CDRII amino acid numbers 50 to 56 and CDRIII amino acid numbers 89 to 97) and the constant region or domain (namely amino acids numbers 108 to 207).

Figure 21 lists amino acids of the heavy chain (HC) of 14D3.

Figure 22 lists amino acids of the heavy chain (HC) of 14D3, showing the variable region or domain (namely amino acid numbers 9 to 120), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 109) and the constant region or domain (namely amino acid numbers 121 to 205).

Figure 23 lists amino acids of the light chain (LC) of 14D3.

Figure 24 lists amino acids of the light chain (LC) of 14D3, showing the variable region or domain (namely amino acid numbers 9 to 107), the CDRs (namely CDR1 amino acid numbers 24 to 34, CDRII amino acid numbers 50 to 56 and CDRIII amino acid numbers 89 to 97) and the constant region or domain (namely amino acids numbers 108 to 207).

Figure 25 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 4D7 as

shown in Figure 9.

Figure 26 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 4D7 as shown in Figure 9, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 10.

Figure 27 lists nucleotide bases encoding amino acids of the light chain (LC) of 4D7 as shown in Figure 11.

Figure 28 lists nucleotide bases encoding amino acids of the light chain (LC) of 4D7 as shown in Figure 11, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 12.

Figure 29 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 16E5 as shown in Figure 13.

Figure 30 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 16E5 as shown in Figure 13, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 14.

Figure 31 lists nucleotide bases encoding amino acids of the light chain (LC) of 16E5 as shown in Figure 15.

Figure 32 lists nucleotide bases encoding amino acids of the light chain (LC) of 16E5 as shown in Figure 15, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 16.

Figure 33 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 17D2 as shown in Figure 17.

Figure 34 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 17D2

as shown in Figure 17, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 18.

Figure 35 lists nucleotide bases encoding amino acids of the light chain (LC) of 17D2 as shown in Figure 19.

Figure 36 lists nucleotide bases encoding amino acids of the light chain (LC) of 17D2 as shown in Figure 19, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 20.

Figure 37 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 14D3 as shown in Figure 21.

Figure 38 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 14D3 as shown in Figure 21, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 22.

Figure 39 lists nucleotide bases encoding amino acids of the light chain (LC) of 14D3 as shown in Figure 23.

Figure 40 lists nucleotide bases encoding amino acids of the light chain (LC) of 14D3 as shown in Figure 23, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 24.

Figure 41 lists amino acids of the heavy chain (HC) of 3B3.

Figure 42 lists amino acids of the heavy chain (HC) of 3B3, showing the variable region or domain (namely amino acid numbers 8 to 112), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 101) and the constant region or domain (namely amino acid numbers 113 to 196).

Figure 43 lists amino acids of the light chain (LC) of 3B3.

Figure 44 lists amino acids of the light chain (LC) of 3B3, showing the variable region or domain (namely amino acid numbers 9 to 111), the CDRs (namely CDR1 amino acid numbers 24 to 38, CDRII amino acid numbers 54 to 60 and CDRIII amino acid numbers 93 to 101) and the constant region or domain (namely amino acids numbers 112 to 211).

Figure 45 lists amino acids of the heavy chain (HC) of 3C7.

Figure 46 lists amino acids of the heavy chain (HC) of 3C7, showing the variable region or domain (namely amino acid numbers 10 to 115), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 104) and the constant region or domain (namely amino acid numbers 116 to 200).

Figure 47 lists amino acids of the light chain (LC) of 3C7.

Figure 48 lists amino acids of the light chain (LC) of 3C7, showing the variable region or domain (namely amino acid numbers 9 to 111), the CDRs (namely CDR1 amino acid numbers 24 to 38, CDRII amino acid numbers 54 to 60 and CDRIII amino acid numbers 93 to 101) and the constant region or domain (namely amino acids numbers 112 to 211).

Figure 49 lists amino acids of the heavy chain (HC) of 2B4.

Figure 50 lists amino acids of the heavy chain (HC) of 2B4, showing the variable region or domain (namely amino acid numbers 9 to 122), the CDRs (namely CDR1 amino acid numbers 31 to 35, CDRII amino acid numbers 50 to 66 and CDRIII amino acid numbers 99 to 111) and the constant region or domain (namely amino acid numbers 123 to 207).

Figure 51 lists amino acids of the light chain (LC) of 2B4.

Figure 52 lists amino acids of the light chain (LC) of 2B4, showing the variable region or

domain (namely amino acid numbers 9 to 112), the CDRs (namely CDR1 amino acid numbers 24 to 39, CDRII amino acid numbers 78 to 82 and CDRIII amino acid numbers 94 to 102) and the constant region or domain (namely amino acids numbers 113 to 212).

Figure 53 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 3B3 as shown in Figure 41.

Figure 54 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 3B3 as shown in Figure 41, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 42.

Figure 55 lists nucleotide bases encoding amino acids of the light chain (LC) of 3B3 as shown in Figure 43.

Figure 56 lists nucleotide bases encoding amino acids of the light chain (LC) of 3B3 as shown in Figure 43, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 44.

Figure 57 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 3C7 as shown in Figure 45.

Figure 58 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 3C7 as shown in Figure 45, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 46.

Figure 59 lists nucleotide bases encoding amino acids of the light chain (LC) of 3C7 as shown in Figure 47.

Figure 60 lists nucleotide bases encoding amino acids of the light chain (LC) of 3C7 as shown in Figure 47, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 48.

Figure 61 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 2B4 as shown in Figure 49.

Figure 62 lists nucleotide bases encoding amino acids of the heavy chain (HC) of 2B4 as shown in Figure 49, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 50.

Figure 63 lists nucleotide bases encoding amino acids of the light chain (LC) of 2B4 as shown in Figure 51.

Figure 64 lists nucleotide bases encoding amino acids of the light chain (LC) of 2B4 as shown in Figure 51, and shows the nucleotide bases encoding the variable region or domain, the CDRs and the constant region or domain as shown in Figure 52.

More specifically, the Figures 1 to 8 illustrate the following:

Figure 1 lists amino acids 1 to 200 of TSH receptors in the above mentioned species, which include the following amino acid sequences employed in the present invention:

amino acids 22 to 91 of a TSH receptor;
amino acids 32 to 41 of a TSH receptor; and
amino acids 36 to 42 of a TSH receptor.

Figure 2 lists nucleotide bases 1 to 300 in the above mentioned species, which include coding regions for the above mentioned amino acid sequences present in Figure 1.

Figure 3 lists amino acids 200 to 300 of TSH receptors in the above mentioned species, which include the following amino acid sequences employed in the present invention:

amino acids 246 to 260 of a TSH receptor; and
amino acids 247 to 260 of a TSH receptor.

Figure 4 lists nucleotide bases 700 to 899 in the above mentioned species, which include coding regions for the above mentioned amino acid sequences present in Figure 3.

Figure 5 lists amino acids 250 to 449 of TSH receptors in the above mentioned species, which include the following amino acid sequences employed in the present invention:

amino acids 260 to 363 of a TSH receptor; and
amino acids 277 to 296 of a TSH receptor.

Figure 6 lists nucleotide bases 750 to 1100 in the above mentioned species, which include coding regions for the above mentioned amino acid sequences present in Figure 5.

Figure 7 lists amino acids 350 to 500 of TSH receptors in the above mentioned species, which include the following amino acid sequences employed in the present invention:

amino acids 380 to 418 of a TSH receptor; and
amino acids 381 to 385 of a TSH receptor.

Figure 8 lists nucleotide bases 1100 to 1299 in the above mentioned species, which include coding regions for the above mentioned amino acid sequences present in Figure 7.

Example 1

(1) Production of mouse monoclonal antibodies to the TSH receptor

BALB/c mice were immunised with a recombinant, highly purified mature form of the TSH receptor expressed in CHO cells. [Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith "Epitope analysis of the human thyrotrophin (TSH) receptor using monoclonal antibodies." Thyroid 2000 10(12): 1051-1059.] Mouse antibodies were also raised by DNA immunisation technique

with full length human TSHR cDNA cloned in pcDNA3.1. MAbs were cloned using standard techniques and IgGs were purified from culture supernatants by affinity chromatography on Protein A Sepharose. The reactivity of MAbs with the TSH receptor was tested by (a) Western blotting with partially purified receptors, (b) inhibition of TSH binding to the TSH receptor, and (c) immunoprecipitation of ^{35}S-labelled TSH receptors produced in an *in vitro* transcription/translation system as described in Y Oda, J Sanders, S Roberts, M Maruyama, R Kato, M Perez, VB Peteresen, N Wedlock, J Furmaniak, B Rees Smith "Binding characteristics of antibodies to the TSH receptor." Journal of Molecular Endocrinology 1998 20: 233-244.

(2) Inhibition of ^{125}I-TSH binding to the TSH receptor

The inhibition of ^{125}I-TSH binding to the TSH receptor was analysed in an assay where, 50µL of detergent solubilised TSH receptor was preincubated with 50µL of MAb purified as described in step (1) for 15 minutes at room temperature before addition of 100µL of ^{125}I-TSH (30,000cpm) followed by incubation at 37°C for one hour. The complexes of ^{125}I-TSH/TSH receptor were precipitated by addition of 2mL 16.5% polyethylene glycol and 25µL healthy blood donor serum, centrifuged at 1500xg for 30 minutes at 4°C, aspirated and the radioactivity of the pellets counted using known techniques.

MAbs termed: 2B4 MAb (at IgG concentration of 5µg/mL), 8E2 Mab (at IgG concentration of 1µg/mL) and 18C5 Mab (at IgG concentration 1mg/mL) showed 76%, 38% and 91% inhibition of TSH binding, respectively. Fab fragments were produced from 2B4 Mab, 8E2 Mab and 18C5 Mab IgGs by digestion with L-cysteine/papain or pepsin, followed by the separation of Fc and Fab on Protein A column.

(3) Epitope recognition by MAbs

Western blotting analysis [Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith "Epitope analysis of the human thyrotrophin (TSH) receptor using monoclonal antibodies." Thyroid 2000 10(12): 1051-

1059.] showed that 2B4 MAb bound to an epitope between amino acid (aa) 380 and 418, 8E2 MAb to an epitope between aa 22 and 91 and 18C5 MAb to an epitope between aa 246 and 260 of the TSH receptor sequence. Analysis with overlapping TSH receptor peptides covering these regions [Y Oda, J Sanders, M Evans, A Kiddie, A Munkley, C James, T Richards, J Wills, J Furmaniak, B Rees Smith "Epitope analysis of the human thyrotrophin (TSH) receptor using monoclonal antibodies." Thyroid 2000 10(12): 1051-1059.] showed that 2B4 MAb reacted with the aa 381 to 385, 8E2 MAb with the aa 36 to 42 and 18C5 MAb with the aa 247 to 260.

(4) Preparation of ^{125}I-labelled TSH receptor

Solubilised preparations of TSH receptor were labelled with ^{125}I by way of ^{125}I-labelled MAB (4E31) reactive with the C-terminal end of the TSH receptor prepared as described in J Sanders, Y Oda, S Roberts, A Kiddie, T Richards, J Bolton, V McGrath, S Walters, D Jaskólski, J Furmaniak, B Rees Smith "The interaction of TSH receptor autoantibodies with ^{125}I-labelled TSH receptor." Journal of Clinical Endocrinology and Metabolism 1999 84(10):3797-3802. Aliquots of ^{125}I-labelled 4E31 $F(ab)_2$ were incubated for 15 minutes at room temperature with solubilised TSH receptor and then used in an immunoprecipitation assay as described in step (5).

(5) Inhibition of TSH receptor autoantibody (TRAb) binding to the TSH receptor by MAbs

The inhibition of TRAb binding to the TSH receptor by MAbs was tested as follows:

10µL of ^{125}I-labelled TSH receptor (30,000cpm) prepared in step (4) was preincubated with 20µL of 2B4 Fab (5 and 10mg/mL) for 15 minutes at room temperature followed by incubation with 20µL of TRAb positive patient serum for one hour at room temperature. 50µL of solid phase Protein A (an anti-human IgG reagent) was then added and incubation continued for one hour at room temperature followed by washing step and centrifugation at 1500xg at 4°C for 30 minutes, aspiration and counting of the

radioactivity of the pellets. Similar experiments were carried out with 8E2 and 18C5 Fabs and the combination of two Fabs together.

Results of Example 1

Results of the inhibition of TRAb binding to the TSH receptor are shown in Table 1.

Example 2

Methods

(1) Production of mouse monoclonal antibodies to the TSH receptor

BALB/C mice were immunised with a recombinant, highly purified mature form of the TSH receptor expressed in CHO cells (Y. Oda, J. Sanders, M. Evans, A. Kiddie, A. Munkley, C. James, T. Richards, J. Wills, J. Furmaniak, B. Rees Smith "Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies" Thyroid 2000 10(12):1051-1059). Mouse antibodies were also raised by DNA immunisation techniques with full length human TSHR cDNA cloned in pRC/CM.1. MAbs were cloned using standard techniques and IgGs were purified from culture supernatants by affinity chromatography on Protein A Sepharose.

$(Fab)_2$ fragments were produced from the purified MAb IgGs by digestion with pepsin followed by chromatography on a protein A affinity column as described in Y. Oda, J. Sanders, S. Roberts, M. Maruyama, R. Kato, M. Perez, VB Petersen, N. Wedlock, J. Furmaniak, B. Rees Smith 1998 "Binding characteristics of antibodies to the TSH receptor". Journal of Molecular Endocrinology **20**: 233-244.

Fab fragments were prepared by digestion of the purified MAbs with papain as described in E. Hendry, G. Taylor, F. Grennan-Jones, A. Sullivan, N. Liddy, J. Godfrey, N. Hayakawa, M. Powell, J. Furmaniak, B Rees Smith 2001 "X-ray crystal structure of a monoclonal antibody that binds to a major autoantigenic epitope on thyroid peroxidase."

Thyroid 11(12): 1091-1099.

The reactivity of MAbs with the TSH receptor was tested by (a) western blotting with partially purified receptors, (b) inhibition of the TSH binding to the TSH receptor and (c) immunoprecipitation of ^{35}S-labelled TSH receptors produced in an *in vitro* transcription/translation system as described in Y. Oda, J. Sanders, S. Roberts, M. Maruyama, R. Kato, M. Perez, VB. Petersen, N. Wedlock, J. Furmaniak, B. Rees Smith "Binding characteristics of antibodies to the TSH receptor" Journal of Molecular Endocrinology 1998 20: 233-244.

(2) Inhibition of ^{125}I TSH binding to the TSH receptor

(a) PEG method for use with detergent solubilised TSHR

The inhibition of ^{125}I TSH binding to detergent solubilised TSH receptor was analysed in an assay where 50 µl of MAb purified as described in Methods (1) above was preincubated with receptor for 15 minutes at room temperature before addition of 100 µl of ^{125}I TSH (30,000 cpm) followed by incubation at 37°C for one hour. The complexes of ^{125}I TSH and TSH receptor were precipitated by addition of 2 mL 16.5% polyethylene glycol and 25 µL healthy blood donor serum, centrifuged at 1500 x g for 30 minutes at 4°C, aspirated and the radioactivity of the pellets counted in a gamma counter.

(b) Method using tubes coated with TSHR

In this procedure, plastic tubes are first coated with a MAb such as 4E31 which binds to a part of the TSHR unrelated to TSH or TRAb binding. Detergent solubilised TSHR preparations are then added, captured by the TSHR MAb and then become immobilised on the tube surface in such a way as to be able to bind TSH or TRAb. In particular the MAb 4E31 reactive with the TSHR C terminus (10 µg/mL $F(ab)_2$ preparation in 0.1 M Na_2CO_3 pH 9.2) was added to plastic tubes (Nunc Maxisorp, 200 µL per tube) and coating allowed to proceed overnight at 4°C. After washing and post-coating (10 mg/mL bovine

serum albumin) the tubes were washed again with assay buffer (10 mM Tris-HCl pH 7.8, 50 mM NaCl, 1 mg/mL bovine serum albumin, 0.1% Triton X-100). 200 µL of a detergent solubilised TSHR preparation was then added and incubated overnight at 4°C followed by aspiration and washing steps. Thereafter, 20 µL of "start" buffer (10 mM Tris-HCl pH 7.8, 50 mM NaCl, bovine serum albumin 1 mg/mL, 6mM NaN_3, 1% Triton X-100) was added to the TSHR coated tubes followed by 100 µL of purified MAb IgG or patient sera and incubated at room temperature for 2 hours with gentle shaking. After aspiration, the tubes were washed twice with 1 mL of assay buffer before addition of 100 µL of ^{125}I TSH (80,000 cpm) and incubation at room temperature for 20-60 min with shaking. The tubes were then washed twice with 1 mL of assay buffer, aspirated and counted in a gamma counter.

(3) Analysis of thyroid stimulating or blocking activities of MAbs.

The ability of MAbs to either stimulate the production of cyclic AMP in isolated porcine thyroid cells (thyroid stimulating activity) or to act as TSH antagonists by blocking TSH stimulation of cyclic AMP (blocking activity) was assessed using reagents from Yamasa Corporation, Tokyo, Japan.

In addition the ability of the MAbs to stimulate production of cyclic AMP in Chinese hamster ovary (CHO) cells expressing human TSHR was analysed as described by M. Kita, L. Ahma, R.C. Marians, H. Vlase, P. Unger, P.N. Graves, T. F. Davies 1999 "Regulation and transfer of a murine model of thyrotropin receptor antibody mediated Graves' disease." Endocrinology 140: 1392-1398.

(4) Binding of ^{125}I-labelled MAbs to the TSHR and effect of TRAb

Purified IgG from two of the MAbs that showed thyroid simulating activity (16E5 and 14D3, table 2) were labelled with ^{125}I followed by separation of unincorporated ^{125}I by filtration on Sephadex G-50 as in (4) in Example 1.

Plastic tubes were coated with TSHR preparations as in 2b above. Thereafter, 100 µL of test serum (from healthy blood donors or from patients with Graves' disease) were added and tubes incubated for 2 hours at room temperature with shaking. After this incubation, the tubes were washed 2 times with assay buffer. Then, 100 µL of ^{125}I-labelled 16E5 or 14D3 IgG (30,000 cpm diluted in 20 mM Tris-HCl pH 7.3, 50 mM NaCl, 1 mg/mL bovine serum albumin, 0.1% Triton X-100) was added to the tubes and incubated for 1 hour at room temperature with shaking. The tubes were then washed twice with the same buffer that was used for diluting ^{125}I-labelled MAbs and counted in a gamma counter.

(5) Binding of TSHR to MAb coated tubes and effect of TRAb

Detergent solubilised TSHR preparations (20 µL) were incubated for 1 hour at room temperature with 100 µL of test serum and 20 µL of start buffer (2b above). 100 µL of this mixture was then added to plastic tubes coated with TSHR MAb (as in 2b above) and incubated for 1 hour with shaking at room temperature. Then the tubes were aspirated and washed twice (2b above) and 100 µL (30,000 cpm) of ^{125}I-labelled C-terminal TSHR MAb 4E31 F(ab)$_2$ preparation labelled with ^{125}I as in 4 above added. After further incubation for 1 hour at room temperature with shaking, the tubes were aspirated, washed twice and the radioactivity counted with a gamma counter.

Oligonucleotide primers were designed using the sequences as described previously (Kettleborough C.A. et al "Optimization of primers for cloning libraries of mouse immunoglobulin genes using the polymerase chain reaction." European Journal of Immunology 1993 23:206-211).

Both sense and antisense primers included additional 5' restriction endonuclease site sequences to facilitate cloning of PCR products. RT-PCR products were cloned into pUC18 DNA prepared by the Qiagen method (Qiagen) and sequenced by the Sanger-Coulson method.

Results of Example 2

(1) Thyroid stimulating activity of the TSHR MAbs is shown in tables 2 and 3. Four of the MAbs (16E5, 14D3, 17D2, and 4D7) were able to stimulate cyclic AMP production in isolated porcine thyroid cells. In addition when Fab fragments from three of these MAbs were tested, all three also stimulated cyclic AMP production (table 2). For comparison a TRAb positive patient serum showed similar levels of stimulation to the MAbs (table 2). Also, TSHR MAb 2B4 which has the ability to inhibit TSH binding to the TSHR strongly did not show thyroid stimulating activity (table 2). Another TSHR MAb and Fab (3B3) did not stimulate cyclic AMP production nor did the Tg MAb Fab 2G2 (table 2).

In a further series of experiments some of the MABS which were able to stimulate porcine thyroid cells (16E5 and 14D3) were tested for their ability to stimulate cyclic AMP production in CHO cells expressing human TSHR (table 3). Similar results were obtained to those observed with porcine thyroid cells.

(2) In the presence of sera from healthy blood donors, ^{125}I-labelled 16E5 bound to TSHR coated tubes is in the range from 23 to 35% of total counts added (table 4). In the presence of sera from patients with Graves' disease (all TRAb positive) the binding of ^{125}I-labelled 16E5 was markedly reduced and was in the range from 1.9 to 7.5% (table 4).

This indicated that Graves' disease patient sera with TRAb activity inhibit the binding of TSHR MAb 16E5 to the TSHR. Further experiments with labelled 16E5 are shown in table 5 where a comparison of the effects of Graves' disease patient sera on (a) ^{125}I-labelled 16E5 to TSHR coated tubes and (b) ^{125}I-labelled TSH binding to TSHR coated tubes. Similar experiments to those shown in table 5 were carried out with ^{125}I-labelled TSHR MAb 14D3 and the results are shown in table 6.

The effects of Graves' disease patient sera on TSHR coated tube binding by ^{125}I-labelled 16E5, 14D3 or TSH were similar with strong inhibition of binding being observed in most cases (tables 5 and 6). In contrast to Graves' disease patient sera, sera from healthy blood donors had little effect on labelled MAb or labelled TSH binding to TSHR coated tubes (tables 5 and 6). Table 7 shows the effect of sera containing autoantibodies other than the TSHR autoantibodies on TSHR coated tube binding by labelled TSH, 16E5 and 14D3. As can be seen from table 7, sera containing autoantibodies to glutamic acid decarboxylase (D1 and D2) or to 21-hydroxylase (A1 and A2) had no effect on TSH or MAb binding. However, the serum G42 from a patient with Graves' disease showed a strong, dose-dependent inhibition of both TSH and MAb binding.

(3) As shown in table 8, plastic tubes coated with MAb 16E5 were able to bind TSHR and this binding was inhibited by Graves' sera containing TSHR autoantibodies. In particular, detection of TSHR binding by the ^{125}I-labelled TSHR MAb 4E31 showed that (a) in the presence of sera from healthy blood donors, labelled 4E31 binding ranged from 13.5-17.8% of total cpm added whereas (b) in the presence of Graves' sera, labelled MAb binding ranged from 1.8-4.8% of total cpm added. Similar results were obtained with plastic tubes coated with MAb 14D3 (table 9).

Conclusions

The results shown in tables 2 - 9 show:

(a) we have produced TSHR MAbs and MAb Fab fragments which can stimulate isolated thyroid cells in a similar way to TRAb in patient sera and in a similar way to TSH. Different MAbs show different degrees of stimulating activity.

(b) these MAbs can be used instead of labelled TSH in assays for TSHR autoantibodies (TRAb).

(c) when the MAbs are coated onto plastic surfaces, they can bind TSHR preparations. This binding is inhibited by TRAb in patient sera, thus providing a new type of TRAb assay.

(d) the ability of the MAbs to stimulate the thyroid means that they are potentially useful as alternatives to TSH in *in vivo* applications.

Example 3

Inhibition of ^{125}I-16E5 Fab binding to solubilised TSH receptor by TSH receptor Mabs

Method

The inhibition of ^{125}I-16E5 Fab binding to detergent solubilised TSH receptor was analysed in an assay where 50µl of Mab IgG (100µg/ml) purified as described above was incubated with receptor for 30 minutes at room temperature before addition of 100µl of ^{125}I-16E5 Fab (30,000 cpm) followed by incubation at room temperature for 2 hours. The complexes of ^{125}I-16E5 Fab and TSH receptor were precipitated by addition of 2ml 16.5% polyethylene glycol and 50µl healthy blood donor serum, centrifuged at 1500 x g for 30 minutes at 4°C, aspirated and the radioactivity of the pellets counted in a gamma counter.

The results are shown in Table 10. From Table 10 it can be seen that Mab 4D7 (which binds to epitope region 246 to 260 and stimulates isolated thyroid cells) quite strongly inhibits labelled 16E5 Fab binding to the TSH receptor (24.2% inhibition). Two other Mabs, 3C7 and 18C5 also quite strongly inhibit 16E5 Fab binding(17 and 15.7% inhibition respectively) and also bind to the epitope region 246 to 260. Weak or no inhibition is observed with the other Mabs. This suggests that epitope region 246 to 260 is involved in 16E5 binding to the TSH receptor. As the other stimulating Mabs 14D3 and 17D2 compete well with 16E5 binding to the TSH receptor as can be seen from Table 10, epitope region 246 to 260 is probably also important for TSH receptor binding by 14D3 and 17D2.

Table 1: Inhibition of binding of TRAb in patient serum (K3) to the TSH receptor by MAb Fabs.

	Serum K3 1/10	
	Labelled TSHR immunoprecipitated (%)	% inhibition
Buffer	17.5	-
2B4 (5mg/ml)	10.1	42
2B4 (10mg/ml)	3.9	77.7
18C5 (5mg/ml)	13.7	21.7
18C5 (10mg/ml)	9.7	44
8E2 (5mg/ml)	15.0	14.3
8E2 (10mg/ml)	13.0	25.7
2B4 + 18C5 (5mg/ml)	5.7	67.4
18C5 + 8E2 (5mg/ml)	12.4	29.1
2B4 + 8E2 (5mg/ml)	8.1	53.7
Unlabelled TSH (2.94mg/ml)	7.8	55.4
2B4+8E2+18C5 (3.3mg/ml)	7.4	57.7

$$\% \text{ inhibition} = 100 - \left(\frac{A}{B} \times 100\right)$$

A = ^{125}I-TSHR (cpm) immunoprecipitated in the presence of test sera and test Mab Fab as a percentage of total cpm of material added to the tube

B = ^{125}I-TSHR (cpm) immunoprecipitated in the presence of test sera and assay buffer as a percentage of total cpm of material added to the tube

The above results show that:

(1) the sequences of the TSH receptor which are involved in the TSH binding are also involved in TRAb binding;

(2) mouse MAb reactive with these sequences can be used effectively to inhibit TRAb binding to the TSH receptor; and

(3) one or more of the MAbs reactive with one or more of the above TSH receptor sequences can be used to detect and measure TRAb.

Table 2: Thyroid stimulating activity of TSHR MAbs tested using isolated porcine thyroid cells

Test sample	Stimulation (%)[1]	Inhibition of TSH binding (%)[2,3]
16E5 IgG 200 µg/ml	466	nt
20 µg/ml	332	83.3
2 µg/ml	269	73.6
0.2 µg/ml	157	nt
0.02 µg/ml	52	nt
14D3 IgG 200 µg/ml	557	nt
20 µg/ml	351	76.4
2 µg/ml	323	61.0
0.2 µg/ml	227	nt
0.02 µg/ml	78	nt
17D2 IgG 200 µg/ml	377	nt
20 µg/ml	207	81.3
2 µg/ml	134	73.7
4D7 IgG 200 µg/ml	259	33[4]
20 µg/ml	31	nt
3B3 IgG[a] 200 µg/ml	34	30.7
20 µg/ml	37	6.1
2B4 IgG[a] 20 µg/ml	100	nt
2 µg/ml	116	69.9
3C7 Fab 1 mg/ml	348	45.2
4D7 Fab 1 mg/ml	512	48.6
16E5 Fab 200 µg/ml	425	53[5]
14D3 Fab 200 µg/ml	648	64[5]
17D2 Fab 200 µg/ml	274	45[5]

3B3 Fab[a] 200 µg/ml	42	66.5[4]
2G2 Fab[6] 1 mg/ml	55	0
200 µg/ml	37	0
TRAb +ve patient dil 1:2	771	65[7]
dil 1:4	530	nt
Pool of healthy blood donor sera	29	0.6
TRAb negative serum	70	0

Table 2 footnotes:

[1] MAb IgG or Fab preparations were diluted in the pool of healthy blood donor sera. Stimulation (%) was calculated as 100x the ratio of: cyclic AMP produced in the presence of test sample to cyclic AMP produced in the presence of a pool of healthy blood donor sera. A stimulation level of > 180% was assessed as positive i.e. this level of stimulation was always greater than that observed by sera from individual healthy blood donors.

[2] Inhibition of TSH binding level of >10% is positive.

[3] Method = coated tube.

[4] Inhibition tested at 250 µg/ml.

[5] Inhibition tested at 10 µg/ml.

[6] 2G2 is a MAb reactive with thyroglobulin i.e. unreactive with the TSHR.

[7] Inhibition with undiluted serum.

[a] 3B3 and 2B4 IgGs act as TSH antagonists i.e. block the ability of TSH to stimulate cyclic AMP production by isolated porcine thyroid cells.

nt = not tested at this concentration.

Table 3: Thyroid stimulating activity of TSHR MAbs tested using CHO cells expressing human TSHR

Test Sample[1]	Stimulation (%)[2]	Inhibition of TSH binding (%)[3,4]
16E5 20µg/ml	850	78.8[5]
14D3 20µg/ml	908	71.8[5]
2B4 20µg/ml	111	84.4
TRAb+ve patient	850	65.0[6]
Pool of healthy blood donor sera	100	0

Table 3 footnotes:

1 All samples were diluted 1:10 prior to addition to cells.

2 Stimulation (%) was calculated as 100x the ratio of: cyclic AMP produced in the presence of test sample to cyclic AMP produced in the presence of a pool of healthy blood donor sera.

3 Inhibition of TSH binding level of >10% is positive.

4 Method = coated tubes

5 Inhibition tested at 10 µg/ml

6 Tested for inhibition undiluted.

Table 4: Binding of ^{125}I-labelled MAb 16E5 to TSHR coated tubes and effect of TRAb in patient sera

Test material[1]	Inhibition of TSH binding (%)[2]	^{125}I-16E5 bound to TSHR coated tubes (%total counts added)
G1	21	5.6
G2	22.7	6.5
G3	24.7	3.5
G4	22.7	6.0
G5	28.1	3.6
G6	29.4	2.5
G7	29.3	5.8
G8	39	1.9
G9	31.9	6.8
G10	34.8	5.4
G11	34.5	3.4
G12	35.3	4.2
G13	35.6	6.2
G14	36.9	2.8
G15	30.3	4.3
G16	35	2.2
G17	47.6	3.9
G18	44.3	3.4
G19	53.5	3.7
G20	59.2	7.5
G21	58.9	4.9
NPS	<14	27.5
NSF 1	<14	23.3
NSF 2	<14	30.2
NSF 3	<14	29.1

NSF 4	<14	22.8
NSF 5	<14	28.9
NSF 6	<14	31.0
NSF 7	<14	29.2
NSF 8	<14	35.3
NSF 9	<14	26.3
NSF 10	<14	25.2

Table 4 Footnotes:

[1] sera G1-G22 are from patients with Graves' disease; sera NSF 1 - NSF 10 are from healthy blood donors;
NPS = pool of healthy blood donor sera

[2] Inhibition of TSH binding >14% is positive; PEG method used.

Table 5: Effect of Graves' disease patient sera on ^{125}I-16E5 binding and ^{125}I-TSH binding to TSHR coated tubes

Test material[1]	^{125}I-16E5 bound to TSHR coated tubes (%total counts added)[2]	Inhibition of ^{125}I-16E5 binding (%)[2,3]	^{125}I-TSH bound to TSHR coated tubes (% total counts added)[4]	Inhibition of ^{125}I TSH binding (%)[3,4]
G23	13.2	44.0	8.9	27.1
G28	5.8	75.4	3.8	68.5
G29	13.3	43.6	8.0	34.4
G30	9.2	61.0	5.3	56.9
G32	11.9	49.6	7.5	38.4
G36	15.5	34.3	10.1	17.5
G38	16.1	31.8	10.0	18.3
G41	17.8	24.6	10.8	11.4
G43	5.9	75.0	4.0	67.2
G44	18.6	21.2	12.4	-ve
G45	5.1	78.4	3.5	71.0
G46	3.8	83.9	2.7	77.9
G47	7.2	69.5	4.3	64.8
G48	6.9	70.8	4.8	60.8
G49	9.1	61.4	6.1	49.6
G50	8.7	63.1	6.3	48.4
G51	11.9	49.6	7.9	35.2
G52	12.3	47.9	7.4	39.0
NSF 4	23.0	2.6	12.5	-ve
NSF 5	25.3	-ve	12.3	-ve
NSF 10	22.4	5.1	12.5	-ve
NSF 16	23.3	1.3	12.0	1.8
NSF 17	24.2	-ve	11.5	5.3
NSF 18	19.9	15.7	11.2	8.0
NSF 20	21.5	8.9	12.3	-ve

NSF 21	23.3	1.3	12.3	-ve
NSF 22	24.5	-ve	12.4	-ve
NSF 26	26.5	-ve	12.8	-ve

Table 5 footnotes:

1 Sera G23-G52 are from patients with Graves' disease;
sera NSF are from healthy blood donors

2 mean binding in the presence of healthy blood donor sera was 23.6% for ^{125}I-16E5.

3 inhibition of binding was calculated using the formula

$$\% \text{ inhibition} = 100 - \left(\frac{A}{B} \times 100\right)$$

where A= binding in the presence of test serum;
B= mean binding in the presence of healthy blood donor sera

4 mean binding in the presence of healthy blood donor sera was 12.2% for ^{125}I-TSH.

Table 6: Binding of ^{125}I-labelled MAb 14D3 to TSHR coated tubes and effect of TRAb in patient sera

Test material[1]	^{125}I-14D3 bound to TSHR coated tubes (%total counts added)[2]	Inhibition of ^{125}I-14D3 binding (%)[2,3]	^{125}I-TSH bound to TSHR coated tubes (% total counts added)[4]	Inhibition of ^{125}I TSH binding (%)[3,4]
G23	13.9	20	8.9	26.6
G24	11.3	35	6.9	43.4
G25	14.1	19	7.2	40.5
G26	7.3	58	2.6	78.3
G27	12.3	29.7	7.3	40.1
G28	8.0	54.4	3.8	68.3
G29	13.2	24.4	8.0	34.0
G30	12.5	28.4	5.3	56.6
G31	9.8	44	4.3	64.3
G32	11.4	34.8	7.5	38.0
G33	12.7	27.2	6.1	49.9
G34	10.9	37.5	7.5	37.8
G35	9.8	43.6	4.3	64.6
G36	13.5	22.8	10.1	16.9
G37	11.9	31.6	9.3	23.4
G38	11.3	35.4	10.0	17.6
G39	12.3	29.5	7.9	34.8
G40	9.8	44.0	7.2	40.9
G41	14.0	19.8	10.8	10.7
NSF 4	17.4	0.3	12.5	-ve
NSF 5	16.5	9.1	12.3	-ve
NSF 10	17.6	-ve	12.5	-ve
NSF 16	17.7	-ve	12.0	1.1
NSF 17	17.0	2.7	11.5	4.6

NSF 18	16.6	8.6	11.2	7.3
NSF 20	18.3	-ve	12.3	-ve
NSF 21	16.8	3.6	12.3	-ve
NSF 22	16.3	6.7	12.4	-ve
NSF 26	18.4	-ve	12.8	-ve

Table 6 footnotes:

1 Sera G23-G41 are from patients with Graves' disease;
sera NSF are from healthy blood donors

2 mean binding in the presence of healthy blood donor sera was 17.4% for ^{125}I-14D3.

3 inhibition of binding was calculated using the formula

$$\% \text{ inhibition} = 100 - \left(\frac{A}{B} \times 100\right)$$

where A= binding in the presence of test serum;
B= mean binding in the presence of healthy blood donor sera

4 mean binding in the presence of healthy blood donor sera was 12.1% for ^{125}I-TSH.

Table 7: Effect of sera from various patients on TSHR coated tube binding by labelled TSH, 16E5 and 14D3

Test sample[1]	%inhibition of binding to TSHR coated tubes [2] using:		
	^{125}I-TSH	^{125}I-16E5	^{125}I-14D3
G42/5	87	71	77
G42/10	82	56	51
G42/20	70	34	24
D1/10	2	3	2
D1/100	-2	3	0
D2/10	1	1	-7
D2/100	-2	0	0
A1/10	-1	3	2
A1/100	-1	3	-1
A2/10	5	3	5
A2/100	1	3	1

Table 7 Footnotes :

[1] Serum G42 is from a patient with Graves' disease;

sera D1 and D2 are from patients with type 1 diabetes mellitus (positive for autoantibodies to glutamic acid decarboxylase)

sera A1 and A2 are from patients with Addison's disease (positive for steroid 21-hydroxylase autoantibodies)

All test samples were diluted in a pool of serum from healthy blood donors and dilution factor shown as /5, /10, /20 or /100

[2] inhibition of binding was calculated using the formula

$$\% \text{ inhibition} = 100 - \left(\frac{A}{B} \times 100\right)$$

where A= binding in the presence of test serum;

B= mean binding in the presence of a pool of healthy blood donor sera

Table 8: Effect of patient sera on binding of TSHR to 16E5 F(ab)$_2$ coated tubes

Test material[1]	^{125}I-4E31 labelled TSHR bound to 16E5 F(ab)$_2$ coated tubes (% total counts added)	Inhibition of TSHR binding[2]	Inhibition of TSH binding (%)[3]
G43	1.8	91.4	72.3
G44	4.8	77.2	45.1
G45	3.0	85.6	71.8
G46	2.0	90.2	83.8
G47	1.8	91.4	75.3
NSF 10	17.8	-15	<14
NSF 17	14.8	4	<14
NSF 21	13.5	12	<14

Table 8 footnotes:

1 Sera G43-G47 are from patients with Graves' disease;
sera NSF are from healthy blood donors

2 inhibition of binding was calculated using the formula

$$\% \text{ inhibition} = 100\text{-} \left(\frac{A}{B} \times 100 \right)$$

where A= binding 4E31 binding in the presence of test serum;
B= mean labelled 4E31 binding for healthy blood donor sera (15.4%)

3 inhibition of TSH binding >14% is positive; PEG method used.

Table 9: Effect of patient sera on binding of the TSHR to 14D3 F(ab)$_2$ coated tubes

Test material[1]	^{125}I-4E31 labelled TSHR bound to 14D3 F(ab)$_2$ coated tubes (% total counts added)	Inhibition of TSHR binding[2]	Inhibition of TSH binding (%)[3]
Serum A	4.0	70	72
Serum B	6.9	49	40
Serum C	3.0	78	85
Serum D	2.6	81	80
NSF 5	15.1	-12	<14
NSF 17	14.6	-9	<14
NSF 21	12.0	10	<14
NSF 23	11.8	12	<14

Table 9 footnotes:

[1] Sera A-D are from patients with Graves' disease;
sera NSF are from healthy blood donors

[2] inhibition of binding was calculated using the formula

$$\% \text{ inhibition} = 100 - \left(\frac{A}{B} \times 100 \right)$$

where A= labelled 4E31 binding in the presence of test serum;
B= mean labelled 4E31 binding for healthy blood donor sera (13.4%)

[3] inhibition of TSH binding >14% is positive; PEG method used.

Table 10 Inhibition of ^{125}I-16E5 Fab binding to TSHR by TSHR MAbs

IgG (100µg/ml)	% inhibition	Epitope region (aa)
16E5	70.4	-
14D3	67.6	-
17D2	69.2	-
2G2	-ve	Thyroglobulin specific
5D6	-ve	22-41
8E2	-ve	22-41
4B5	7.1	22-41
10C4	-ve	37-56
10D5	-ve	37-71
4D2	-ve	37-71
2E2	-ve	52-71
1D6	-ve	202-221
7B5	-ve	202-221
16B6	-ve	202-221
3C3	11.2	202-236
4B4	-ve	217-236
4E4	-ve	217-236
8D3	-ve	217-236
6D7	-ve	217-236
18C5	15.7	246-260
3C7	17	246-260
4D7	24.2	246-260
3B3	8.8	277-296
5B5	-ve	307-326
4E6	-ve	307-326
6E2	-ve	322-341
9C2	-ve	322-341
6B4	-ve	337-356
3E4	-ve	337-371
3F3	-ve	352-371
3B2	-ve	352-371
7C2	-ve	367-386
2B4	-ve	381-385
3E6	5.4	381-385
8E3	4.8	381-385
7C4	-ve	381-385
1D5	4.2	381-385
4E2	-ve	381-385
3D3	-ve	382-401
2C4	-ve	382-401
10C2	-ve	382-401
7E5	-ve	382-401

CLAIMS

1. For use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact with said polypeptide sequence, so as to enable said diagnosis or therapy.

2. Use according to claim 1, wherein said polypeptide sequence comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments;

3. Use according to claim 1, wherein said polypeptide sequence comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments.

4. For use in diagnosis or therapy of autoimmune disease associated with an immune reaction to a TSH receptor, a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7,

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact with said polypeptide sequence, so as to enable said diagnosis or therapy.

5. Use according to claim 4, wherein said polypeptide sequence comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments.

6. Use according to claim 4, wherein said polypeptide sequence comprises, consists

of or consists essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments.

7. Use according to any of claims 1 to 6, wherein said polypeptide sequence comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor.

8. Use according to any of claims 1 to 7, employing:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments;

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact with said polypeptide sequences, so as to enable said diagnosis or therapy.

9. Use according to claim 8, which further employs:

(iii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor, or variants, analogs or derivatives of such fragments.

10. Use according to any of claims 1 to 9, which employs:

(i) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more TSH receptor epitopes with which autoantibodies and / or

lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments; and

(ii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments;

wherein autoantibodies and / or lymphocytes produced in response to a TSH receptor interact with said polypeptide sequences, so as to enable said diagnosis or therapy.

11. Use according to claim 10, which further employs:

(iii) a polypeptide sequence comprising, consisting of or consisting essentially of part or all of the primary structural conformation of one or more further TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said

polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or variants, analogs or derivatives of such fragments.

12. Use according to any of claims 1 to 11, wherein said polypeptide sequence or sequences interact with autoantibodies produced in response to the TSH receptor, so as to enable said diagnosis or therapy.

13. Use according to any of claims 1 to 11, wherein said polypeptide sequence interacts with lymphocytes produced in response to the TSH receptor, so as to enable said diagnosis or therapy.

14. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor.

15. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or

fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7

16. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor.

17. A TSH receptor epitope comprising, consisting of or consisting essentially of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

18. A TSH receptor epitope comprising, consisting of or consisting essentially of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of

polypeptide sequence comprising, consisting of or consisting essentially of the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or variants, analogs or derivatives of such fragments.

12. Use according to any of claims 1 to 11, wherein said polypeptide sequence or sequences interact with autoantibodies produced in response to the TSH receptor, so as to enable said diagnosis or therapy.

13. Use according to any of claims 1 to 11, wherein said polypeptide sequence interacts with lymphocytes produced in response to the TSH receptor, so as to enable said diagnosis or therapy.

14. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor.

15. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or

fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7

16. One or more TSH receptor epitopes with which autoantibodies and / or lymphocytes produced in response to a TSH receptor interact, said one or more TSH receptor epitopes comprising, consisting of or consisting essentially of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor.

17. A TSH receptor epitope comprising, consisting of or consisting essentially of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

18. A TSH receptor epitope comprising, consisting of or consisting essentially of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of

such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

19. A TSH receptor epitope comprising, consisting of or consisting essentially of amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

20. One or more TSH receptor epitopes according to any of claims 14 to 19, which interacts with autoantibodies produced in response to a TSH receptor.

21. One or more TSH receptor epitopes according to any of claims 14 to 19, which interacts lymphocytes produced in response to a TSH receptor.

22. A polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

with which autoantibodies and / or lymphocytes produced in response to a TSH

receptor can interact, with the exception of a full length TSH receptor.

23. A polypeptide according to claim 22, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments.

24. A polypeptide according to claim 22, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments.

25. A polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 22 to 91 of a TSH receptor;
amino acid numbers 246 to 260 of a TSH receptor;
amino acid numbers 260 to 363 of a TSH receptor; and
amino acid numbers 380 to 418 of a TSH receptor;

as depicted in any one of the amino acid sequences of any of Figures 1, 3, 5 and 7, with which autoantibodies and / or lymphocytes produced in response to a TSH

receptor can interact, with the exception of a full length TSH receptor.

26. A polypeptide according to claim 25, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments.

27. A polypeptide according to claim 25, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments.

28. A polypeptide according to any of claims 22 to 27, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of one or more of the following, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments:

amino acid numbers 32 to 41 of a TSH receptor;
amino acid numbers 36 to 42 of a TSH receptor;
amino acid numbers 247 to 260 of a TSH receptor;
amino acid numbers 277 to 296 of a TSH receptor; and
amino acid numbers 381 to 385 of a TSH receptor;

with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

29. A polypeptide with which autoantibodies and / or lymphocytes produced in

response to a TSH receptor can interact and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, with the exception of a full length TSH receptor.

30. A polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, with the exception of a full length TSH receptor.

31. A polypeptide with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact and which comprises, consists of or consists essentially of part or all of the primary structural conformation of one or more epitopes of a TSH receptor with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, which polypeptide comprises, consists of or consists essentially of the primary structural conformation of amino acid numbers 247 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or

derivatives of such fragments with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact, with the exception of a full length TSH receptor.

32. A polypeptide according to any of claims 22 to 31, which polypeptide comprises, consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact; and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

33. A polypeptide according to claim 32, which further comprises:

(iii) the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

34. A polypeptide according to any of claims 22 to 33, which polypeptide comprises,

consists of or consists essentially of:

(i) the primary structural conformation of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or one or more variants, analogs, derivatives or fragments of amino acid numbers 277 to 296 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 5, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact; and

(ii) the primary structural conformation of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or one or more variants, analogs, derivatives or fragments of amino acid numbers 246 to 260 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 3, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

35. A polypeptide according to claim 34, which further comprises:

(iii) the primary structural conformation of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or one or more variants, analogs, derivatives or fragments of amino acid numbers 381 to 385 of a TSH receptor as depicted in any one of the amino acid sequences of Figure 7, or variants, analogs or derivatives of such fragments, with which autoantibodies and / or lymphocytes produced in response to a TSH receptor can interact.

36. A polynucleotide comprising

(i) a nucleotide sequence encoding a polypeptide according to any of claims 22 to 35;

(ii) a nucleotide sequence encoding a polypeptide according to any of claims 22 to 35, which polypeptide comprises an amino acid sequence or sequences of specified amino acid numbers of a TSH receptor which is or are defined by reference to any of Figures 1, 3, 5 and 7;

(iii) a nucleotide sequence encoding a polypeptide of (ii), which nucleotide sequence comprises nucleotide bases encoding the above mentioned specified amino acid numbers of a TSH receptor which are defined by reference to any of Figures 1, 3, 5, and 7, and which nucleotide bases are defined by reference to any of Figures 2, 4, 6 and 8;

(iv) a nucleotide sequence differing from the sequence of (iii) in codon sequence due to the degeneracy of the genetic code;

(v) a nucleotide sequence comprising an allelic variation of the sequence of (iii);

(vi) a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v); or

(vii) a nucleotide sequence which hybridizes under stringent conditions to any of the sequences of (i), (ii), (iii), (iv), (v) or (vi).

37. A biologically functional plasmid which carries a polynucleotide according to claim 36, and which is capable of introducing the polynucleotide into the genome of a host organism.

38. A method of screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing either (i) said sample of body fluid from said subject or (ii) lymphocytes isolated from said sample;

(b) contacting said sample or isolated lymphocytes with a polypeptide according to any of claims 22 to 35, so as to permit said polypeptide to interact with autoantibodies, or lymphocytes, produced in response to a TSH receptor, and present in, or isolated from, said sample; and

(c) monitoring the degree, or effect, of interaction of said polypeptide with either said autoantibodies, or said lymphocytes, produced in response to a TSH receptor and present in, or isolated from, said sample, thereby providing an indication of the presence of said autoantibodies, or said lymphocytes, in said sample, or isolated from said sample.

39. A method according to claim 38, of screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from said subject.

40. A method according to claim 38, of screening for lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from said subject.

41. A method according to claim 38, which comprises directly monitoring interaction of autoantibodies to a TSH receptor present in the sample of body fluid from the subject and a polypeptide according to any of claims 22 to 35, employing non-competitive sandwich type assay techniques.

42. A method according to claim 38, which employs at least one competitor capable

of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to any of claims 22 to 35.

43. A method according to claim 42, wherein said competitor comprises one or more antibodies.

44. A method of screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor, and

(ii) at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to any of claims 22 to 35,

so as to permit said full length TSH receptor to interact with either autoantibodies to a TSH receptor present in said sample, or said competitor; and

(c) monitoring the interaction of said full length TSH receptor with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

45. A kit for screening for autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from a subject suspected of

suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a polypeptide according to any of claims 22 to 35;

(b) means for contacting either (i) a sample of body fluid obtained from said subject, or (ii) lymphocytes isolated from a sample of body fluid obtained from said subject, with said polypeptide according to any of claims 22 to 35, so as to permit said polypeptide to interact with autoantibodies, or lymphocytes, produced in response to a TSH receptor, and present in, or isolated from, said sample; and

(c) means for monitoring the degree, or effect, of interaction of said polypeptide with either said autoantibodies, or said lymphocytes, produced in response to a TSH receptor and present in, or isolated from, said sample, thereby providing an indication of the presence of said autoantibodies, or lymphocytes, in said sample or isolated from said sample.

46. A kit according to claim 45, for screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from said subject.

47. A kit according to claim 45, for screening for lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from said subject.

48. A kit according to claim 45, comprising means for directly monitoring interaction of autoantibodies to a TSH receptor present in the sample of body fluid from the subject and a polypeptide according to any of claims 22 to 35, comprising non-competitive sandwich type assay means.

49. A kit according to claim 45, further comprising at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to any of claims 22 to 35 .

50. A kit according to claim 49, wherein said competitor comprises one or more antibodies.

51. A kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor;

(b) at least one competitor capable of competing with autoantibodies to a TSH receptor in the interaction thereof with a polypeptide according to any of claims 22 to 35;

(c) means for contacting said sample of body fluid from said subject, said full length TSH receptor and said competitor, so as to permit said full length TSH receptor to interact with either autoantibodies to a TSH receptor present in said sample, or said competitor; and

(d) means for monitoring the interaction of said full length TSH receptor with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

52. A method of diagnosing the likely onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in a subject suspected of suffering from, susceptible to, having or recovering from, autoimmune disease associated with an immune reaction to a TSH receptor, the method comprising

detecting autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid from the subject according to any of claims 38 to 44, and whereby the detected autoantibodies and / or lymphocytes can provide a diagnosis of the likely onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in the subject.

53. A method of delaying or preventing the onset of autoimmune disease associated with an immune reaction to a TSH receptor in an animal subject suspected of suffering from, susceptible to or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, which method comprises initially detecting autoantibodies or lymphocytes indicative of the onset or presence of autoimmune disease associated with an immune reaction to a TSH receptor in a sample of body fluid obtained from the subject according to any of claims 38 to 44, thereby providing a diagnosis of the likely onset of autoimmune disease associated with an immune reaction to a TSH receptor in the subject, and thereafter therapeutically treating the subject so as to delay the onset and / or prevent autoimmune disease associated with an immune reaction to a TSH receptor.

54. A polypeptide according to any of claims 22 to 35, for use in the therapeutic treatment of autoimmune disease associated with an immune reaction to a TSH receptor.

55. A pharmaceutical composition comprising a polypeptide according to any of claims 22 to 35, together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

56. A polypeptide according to any of claims 22 to 35, for use in the manufacture of a medicament for the treatment of Graves' disease.

57. One or more therapeutic agents identified as providing a therapeutic effect by

interaction with amino acids comprising part or all of the primary conformation of amino acids of one or more epitopes of a TSH receptor according to any of claims 14 to 21.

58. A method of treating autoimmune disease associated with an immune reaction to a TSH receptor in a subject, which method comprises initially detecting autoantibodies or lymphocytes produced in response to a TSH receptor in a sample of body fluid obtained from the subject according to any of claims 38 to 44, thereby providing a diagnosis of autoimmune disease in the subject, and administering to the subject a therapeutically effective amount of at least one therapeutic agent effective in the treatment of such autoimmune disease.

59. A method according to claim 58, wherein said therapeutic agent comprises a polypeptide according to any of claims 22 to 35.

60. A method of treating autoimmune disease associated with an immune reaction to a TSH receptor in a subject, which method comprises administering to the subject a therapeutically effective amount of a therapeutic agent identified as providing a therapeutic effect by interaction with amino acids comprising part or all of the primary conformation of amino acids of one or more epitopes of a TSH receptor according to any of claims 14 to 21.

61. A binding partner for a TSH receptor capable of interacting with amino acid numbers 277 to 296 of a TSH receptor.

62. A binding partner according to claim 61, which is a monoclonal or recombinant antibody.

63. A binding partner for a TSH receptor, which binding partner is capable of binding to a TSH receptor so as to stimulate the TSH receptor, which binding partner does not comprise TSH or naturally produced autoantibodies to the TSH receptor.

64. A binding partner according to claim 63, which is a monoclonal antibody or fragment thereof.

65. A binding partner according to claim 64, which is a recombinant antibody or fragment thereof.

66. A binding partner according to any of claims 63 to 65, which interacts with one or more epitope regions according to any of claims 14 to 21.

67. A binding partner for a TSH receptor, which binding partner is capable of binding to the TSH receptor so as to stimulate the TSH receptor and which comprises:

an antibody V_H domain selected from the group consisting of:

V_H domains as shown in any one of Figures 10, 14, 18, 22, 42, 46 or 50, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 10, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 14, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 18, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 22, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 42, a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 46, and a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 50; and / or

an antibody V_L domain selected from the group consisting of:

V_L domains as shown in any one of Figures 12, 16, 20, 24, 44, 48 or 52, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 12, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 16, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 20, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 24, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 44, a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 48, and a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 52.

68. A binding partner according to claim 67, comprising an antibody V_H domain as shown in Figure 10 paired with an antibody V_L domain as shown in Figure 12 to provide an antibody binding site, comprising both these V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 14 paired with an antibody V_L domain as shown in Figure 16 to provide an antibody binding site, comprising both these V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 18 paired with an antibody V_L domain as shown in Figure 20 to provide an antibody binding site comprising both these V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 22 paired with an antibody V_L domain as shown in Figure 24 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 42 paired with an antibody V_L domain as shown in Figure 44 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 46 paired with an antibody

V_L domain as shown in Figure 48 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor; or comprising an antibody V_H domain as shown in Figure 50 paired with an antibody V_L domain as shown in Figure 52 to provide an antibody binding site comprising both V_H and V_L domains for a TSH receptor.

69. A binding partner according to claim 67, which comprises:

an antibody V_H domain comprising:

a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 10, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 14, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 18, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 22, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 42, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 46, or a V_H domain comprising one or more V_H CDRs with an amino acid sequence corresponding to a V_H CDR as shown in Figure 50; and / or

an antibody V_L domain comprising:

a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 12, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 16, or a V_L domain

comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 20, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 24, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 44, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 48, or a V_L domain comprising one or more V_L CDRs with an amino acid sequence corresponding to a V_L CDR as shown in Figure 52.

70. A further binding partner capable of binding to the TSH receptor so as to stimulate the TSH receptor and which further binding partner can compete for binding to the TSH receptor with any specific binding partner according to any of claims 63 to 69, which further binding partner does not comprise TSH or autoantibodies to a TSH receptor.

71. A polynucleotide comprising:

(i) a nucleotide sequence as shown in any of Figures 25 to 40, or 53 to 64; or parts of such sequences as shown in Figures 26, 28, 30, 32, 34 ,36, 38, 40, 54, 56, 58, 60, 62, or 64, encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR as shown in any of Figures 10, 12, 14, 16, 18, 20, 22, 24, 42, 44, 46, 48, 50 or 52;

(ii) a nucleotide sequence encoding a binding partner according to any of claims 63 to 70, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR of a binding partner according to any of claims 63 to 70;

(iii) a nucleotide sequence encoding a binding partner having a primary

structural conformation of amino acids as shown in any of Figures 9 to 24 or 41 to 52, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR as shown in any of Figures 10, 12, 14, 16, 18, 20, 22, 24, 42, 44, 46, 48, 50 or 52;

(iv) a nucleotide sequence differing from any sequence of (i) in codon sequence due to the degeneracy of the genetic code;

(v) a nucleotide sequence comprising an allelic variation of any sequence of (i);

(vi) a nucleotide sequence comprising a fragment of any of the sequences of (i), (ii), (iii), (iv) or (v);

(vii) a nucleotide sequence differing from the any sequence of (i) due to mutation, deletion or substitution of a nucleotide base and encoding a binding partner according to any of claims 63 to 70, or encoding an amino acid sequence of an antibody V_H domain, an antibody V_L domain or CDR of a binding partner according to any of claims 63 to 70.

72. A biologically functional vector system which carries a polynucleotide according to claim 71 and which is capable of introducing the polynucleotide into the genome of a host organism.

73. A method of screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor, and

(ii) one or more binding partners according to any of claims 63 to 70;

so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding partners; and

(c) monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

74. A method according to claim 73, wherein said polypeptide is according to any of claims 22 to 35.

75. A method according to claim 73, wherein said one or more epitopes of a TSH receptor are according to any of claims 14 to 21.

76. A method according to any of claims 73 to 75, which comprises providing labelling means for said one or more binding partners according to any of claims 63 to 70.

77. A method of screening for autoantibodies produced in response to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from,

susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said method comprising:

(a) providing said sample of body fluid from said subject;

(b) contacting said sample with

(i) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor, and

(ii) one or more binding members for a TSH receptor;

so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding members; and

(c) monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample;

wherein said one or more binding members are directly or indirectly immobilised to a surface either prior to, or after step (b).

78. A method according to claim 77, wherein said binding member comprises a binding partner as defined in any of claims 63 to 70.

79. A method according to claim 77, wherein said polypeptide is according to any of claims 22 to 35.

80. A method according to claim 77, wherein said one or more epitopes of a TSH receptor are according to any of claims 14 to 21.

81. A method according to claim 77, which comprises providing labelling means for said TSH receptor, said one or more epitopes thereof or said polypeptide.

82. A kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor;

(b) one or more binding partners according to any of claims 63 to 70;

(c) means for contacting said sample of body fluid from said subject, said TSH receptor, said one or more epitopes thereof or said polypeptide, and said one or more binding partners, so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding partners; and

(d) means for monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

83. A kit according to claim 82, wherein said polypeptide is according to any of claims 22 to 35.

84. A kit according to claim 82, wherein said one or more epitopes of a TSH receptor are according to any of claims 14 to 21.

85. A kit according to claim 82, comprising labelling means for said one or more binding partners according to any of claims 63 to 70.

86. A kit for screening for autoantibodies to a TSH receptor in a sample of body fluid obtained from a subject suspected of suffering from, susceptible to, having or recovering from autoimmune disease associated with an immune reaction to a TSH receptor, said kit comprising:

(a) a full length TSH receptor, one or more epitopes thereof or a polypeptide comprising one or more epitopes of a TSH receptor;

(b) one or more binding members for a TSH receptor;

(c) means for contacting said sample of body fluid from said subject, said TSH receptor, said one or more epitopes thereof or said polypeptide, and said one or more binding members, so as to permit said TSH receptor, said one or more epitopes thereof or said polypeptide, to interact with either autoantibodies to a TSH receptor present in said sample, or said one or more binding members;

(d) means for directly or indirectly immobilising said one or more binding members to a surface, either before or after contacting said one or more binding members with said sample of body fluid from said subject and said TSH receptor, said one or more epitopes thereof or said polypeptide; and

(e) means for monitoring the interaction of said TSH receptor, said one or more epitopes thereof or said polypeptide, with said autoantibodies present

in said sample, thereby providing an indication of the presence of said autoantibodies to a TSH receptor in said sample.

87. A kit according to claim 86, wherein said binding member comprises a binding partner as defined in any of claims 63 to 70.

88. A kit according to claim 86, wherein said polypeptide is according to any of claims 22 to 35.

89. A kit according to claim 86, wherein said one or more epitopes of a TSH receptor are according to any of claims 14 to 21.

90. A kit according to claim 86, comprising labelling means for said TSH receptor, said one or more epitopes thereof or said polypeptide.

91. A method of treating autoimmune disease associated with an immune reaction to a TSH receptor in a subject, comprising administering to said subject a therapeutically effective amount of a specific binding partner as defined in any of claims 63 to 70.

92. A pharmaceutical composition comprising a specific binding partner as defined in any of claims 63 to 70, togther with one or more pharmaceutically acceptable carriers, diluents or excipients therefor.

93. Use of a specific binding partner as defined in any of claims 63 to 70, in the manufacture of a medicament for use in stimulating thyroid tissue, or tissue containing a TSH receptor.

94. Use of a specific binding partner as defined in any of claims 63 to 70, in the manufacture of a medicament for treatment of thyroid cancer.

95. A method of stimulating thyroid tissue, and / or tissue containing a TSH receptor, which method comprises administering to a patient in need of such stimulation a diagnostically or therapeutically effective amount of a binding partner as defined in any of claims 63 to 70.

96. In combination a binding partner as defined in any of claims 63 to 70, together with one or more further agents capable of stimulating thyroid tissue, and / or tissue containing a TSH receptor, for simultaneous, separate or sequential use in stimulating thyroid tissue, and / or tissue containing a TSH receptor.

97. A combination according to claim 96, wherein said one or more further agents comprise recombinant human TSH and / or one or more variants, analogs, derivatives or fragments thereof, or variants, analogs or derivatives of such fragments.

98. A combination according to claim 96, wherein said one or more further agents acts independently of binding to a TSH receptor.

```
     MRPTPLLQLALLALPRSLGGKGCPSPPCECHQEDDFRVT   Majority

1    MRPADLLQLVLLLDLPRDLGGMGCSSPPCECHQEEDFRVT  HTSHR.PRO
1    MSLTPLLQLALVLALPRSLRGKGCPSPPCECHQEDDFRVT  PTSHR.PRO
1    MRPTPLLRLALFLVLPSSLGGERCPSPPCECRQEDDFRVT  BTSHR.PRO
1    MRQTPLLQLALLLSLPRSLGGKGCPSPPCECHQEDDFRVT  CTSHR.PRO
1    MRPPPLLHLALLLALPRSLGGKGCPSPPCECHQEDDFRVT  DTSHR.PRO
1    MRPGSLLLLVLLLALSRSLRGKECASPPCECHQEDDFRVT  MTSHR.PRO
1    MRPGSLLQLTLLLALPRSLWGRGCTSPPCECHQEDDFRVT  RTSHR.PRO
1    MRPTPLLRLALLLVLPSSLWGERCPSPPCECRQEDDFRVT  STSHRP.PRO

     CKDIHRIPSLPPSTQTLKFIETHLKTIPSRAFSNLPNISR  Majority

41   CKDIQRIPSLPPSTQTLKLIETHLRTIPSHAFSNLPNISR  HTSHR.PRO
41   CKDIHSIPPLPPNTQTLKFIETHLKTIPSRAFSNLPNISR  PTSHR.PRO
41   CKDIQSIPSLPPSTQTLKFIETHLKTIPSRAFSNLPNISR  BTSHS.PRO
41   CKDIHRIPSLPPSTQTLKFIETHLKTIPSRAFSNLPNISR  CTSHR.PRO
41   CKDIHRIPTLPPSTQTLKFIETQLKTIPSRAFSNLPNISR  DTSHR.PRO
41   CKELHRIPSLPPSTQTLKLIETHLKTIPSLAFSSLPNISR  MTSHR.PRO
41   CKELHQIPSLPPSTQTLKLIETHLKTIPSLAFSSLPNISR  RTSHS.PRO
41   CKDIQRIPSLPPSTQTLKFIETHLKTIPSRAFSNLPNISR  STSHRP.PRO

     IYLSIDATLQQLESHSFYNLSKMTHIEIRNTRSLTYIDPG  Majority

81   IYVSIDVTLQQLESHSFYNLSKVTHIEIRNTRNLTYIDPD  HTSHR.PRO
81   IYLSIDATLQQLESQSFYNLSKMTHIEIRNTRSLTYINPG  PTSHR.PRO
81   IYLSIDATLQQLESHSFYNLSKVTHIEIRNTRSLTYIDSG  BTSHR.PRO
81   IYLSIDATLQRLESHSFYNLSKMTHIEIRNTRSLTYIDPG  CTSHR.PRO
81   IYLSIDATLQRLESHSFYNLSKMTHIEIRNTRSLTSIDPD  DTSHR.PRO
81   IYLSIDATLQRLEPHSFYNLSKMTHIEIRNTRSLTYIDPD  MTSHR.PRO
81   IYLSIDATLQRLEPHSFYNLSKMTHIEIRNTRSLTYIDPD  RTSHR.PRO
81   IYLSIDATLQQLESHSFYNLSKVTHIEIRNTRSLTYIDSG  STSHRP.PRO

     ALKELPLLKFLGIFNTGLRVFPDLTKVYSTDVFFILEITD  Majority

121  ALKELPLLKFLGIFNTGLKMFPDLTKVYSTDIFFILEITD  HTSHR.PRO
121  ALKDLPLLKFLGIFNTGLRIFPDLTKVYSTDVFFILEITD  PTSHR.PRO
121  ALKELPLLKFLGIFNTGLRVFPDLTKIYSTDVFFILEITD  BTSHR.PRO
121  ALKELPLLKFLGIFNTGLGVFPDLTKVYSTDVFFILEITD  CTSHR.PRO
121  ALKELPLLKFLGIFNTGLGVFPDVTKVYSTDVFFILEITD  DTSHR.PRO
121  ALTELPLLKFLGIFNTGLRIFPDLTKIYSTDIFFILEITD  MTSHR.PRO
121  ALTELPLLKFLGIFNTGLRIFPDLTKIYSTDVFFILEITD  RTSHR.PRO
121  ALKELPLLKFLGIFNTGLRVFPDLTKIYSTDVFFILEITD  STSHRP.PRO

     NPYMTSIPANAFQGLCNETLTLKLYNNGFTSIQGHAFNGT  Majority

161  NPYMTSIPVNAFQGLCNETLTLKLYNNGFTSVQGYAFNGT  HTSHR.PRO
161  NPYMTSIPANAFQGLCNETLTLKLYNNGFTSVQGHAFNGT  PTSHR.PRO
161  NPYMTSIPANAFQGLCNETLTLKLYNNGFTSIQGHAFNGT  BTSHR.PRO
161  NPYMTSIPANAFQGLCNETLTLKLYNNGFTSIQGHAFNGT  CTSHR.PRO
161  NPYMASIPANAFQGLCNETLTLKLYNNGFTSIQGHAFNGT  DTSHR.PRO
161  NPYMTSVPENAFQGLCNETLTLKLYNNGFTSVQGHAFNGT  MTSHR.PRO
161  NPYMTSVPENAFQGLCNETLTLKLYNNGFTSIQGHAFNGT  RTSHR.PRO
161  NPYMTSVPANAFQGLSNETLTLKLYNNGFTSIQGHAFNGT  STSHRP.PRO
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FIG. 1

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     ATGAGGCCGACGCCCCTGCTGCAGCTGGCGCTGCTTCTCG  Majority

   1 ATGAGGCAGACGCCCCTGCTGCAGCTGGCGTTACTTCTCT  CAT.SEQ
   1 ATGCGGCCGACGCCCCTCCTGCGGCTGGCGCTGTTTCTGG  COW.SEQ
   1 ATGAGGCCGCCGCCCCTGCTGCACCTGGCGCTGCTTCTCG  DOG.SEQ
   1 ATGAGGCCAGGGTCCCTGCTGCTGCTTGTTCTGCTGCTCG  MOUSE.SEQ
   1 ATGAGTCTGACGCCCCTGTTGCAGCTGGCGCTCGTTCTCG  PTSHR.SEQ
   1 ATGAGGCCAGGGTCCCTGCTCCAGCTCACTCTGCTGCTCG  RAT.SEQ
   1 ATGCGGCCGACGCCCCTCCTGCGGTTGGCGCTGCTTCTGG  SHEEP.SEQ
   1 ATGAGGCCGGCGGACTTGCTGCAGCTGGTGCTGCTGCTCG  HTSHR.SEQ

     CCCTGCCCAGGAGCCTGGGGGGGAAGGGGTGTCCGTCTCC  Majority

  41 CCCTGCCCAGGAGCCTGGGGGGGAAAGGGTGTCCGTCTCC  CAT.SEQ
  41 TCCTGCCCAGCAGCCTCGGTGGGGAGAGGTGTCCGTCTCC  COW.SEQ
  41 CCCTGCCCAGGAGCCTGGGGGGGAAGGGGTGTCCTTCTCC  DOG.SEQ
  41 CCCTGTCCAGGAGCCTGCGGGGCAAAGAGTGTGCGTCTCC  MOUSE.SEQ
  41 CCCTGCCCAGGAGCCTCAGGGGGAAAGGGTGTCCGTCTCC  PTSHR.SEQ
  41 CCCTGCCCAGGAGCCTCTGGGGCAGAGGGTGTACTTCTCC  RAT.SEQ
  41 TCCTGCCCAGCAGCCTCTGGGGGGAGAGGTGTCCGTCTCC  SHEEP.SEQ
  41 ACCTGCCCAGGGACCTGGGCGGAATGGGGTGTTCGTCTCC  HTSHR.SEQ

     GCCCTGCGAGTGCCACCAGGAGGACGACTTCAGAGTCACC  Majority

  81 GCCCTGCGAGTGTCACCAGGAAGATGACTTCAGAGTCACC  CAT.SEQ
  81 GCCCTGCGAATGCCGCCAGGAGGACGACTTCAGAGTCACC  COW.SEQ
  81 CCCCTGTGAGTGCCACCAGGAGGATGACTTCAGAGTCACC  DOG.SEQ
  81 ACCCTGTGAGTGTCACCAGGAGGACGACTTCAGAGTCACC  MOUSE.SEQ
  81 GCCCTGCGAATGCCACCAGGAGGACGACTTCAGAGTCACC  PTSHR.SEQ
  81 ACCCTGCGAATGCCACCAGGAGGACGACTTCAGAGTCACC  RAT.SEQ
  81 GCCCTGCGAATGCCGCCAGGAGGACGACTTCAGAGTCACC  SHEEP.SEQ
  81 ACCCTGCGAGTGCCATCAGGAGGAGGACTTCAGAGTCACC  HTSHR.SEQ

     TGCAAGGATATCCACCGCATCCCCAGCTTACCGCCCAGCA  Majority

 121 TGCAAGGATATTCACCGTATCCCCAGCCTACCGCCCAGCA  CAT.SEQ
 121 TGCAAGGACATCCAGAGCATCCCTAGCTTACCCCCCAGCA  COW.SEQ
 121 TGCAAGGATATCCACCGCATCCCCACCCTACCACCCAGCA  DOG.SEQ
 121 TGCAAGGAGCTCCACCGAATCCCCAGCCTGCCGCCCAGCA  MOUSE.SEQ
 121 TGCAAGGATATCCACAGCATCCCCCCCTTACCACCCAATA  PTSHR.SEQ
 121 TGCAAGGAACTCCACCAAATCCCCAGCCTACCGCCCAGCA  RAT.SEQ
 121 TGCAAGGACATCCAGCGCATCCCTAGCTTACCCCCCAGCA  SHEEP.SEQ
 121 TGCAAGGATATTCAACGCATCCCCAGCTTACCGCCCAGTA  HTSHR.SEQ

     CGCAGACTCTGAAGTTTATAGAGACTCATCTGAAAACCAT  Majority

 161 CGCAGACTCTGAAATTTATAGAGACTCATCTGAAAACCAT  CAT.SEQ
 161 CGCAGACCCTGAAGTTTATAGAGACTCATCTGAAAACCAT  COW.SEQ
 161 CGCAGACTCTGAAGTTTATAGAGACTCAGCTGAAAACCAT  DOG.SEQ
 161 CCCAGACTCTGAAGCTCATCGAGACTCATCTGAAGACCAT  MOUSE.SEQ
 161 CTCAGACACTAAAGTTTATAGAGACTCATCTGAAAACCAT  PTSHR.SEQ
 161 CCCAGACTCTGAAGCTCATCGAGACTCACCTGAAGACCAT  RAT.SEQ
 161 CGCAGACCCTGAAGTTTATAGAGACTCATCTGAAAACCAT  SHEEP.SEQ
 161 CGCAGACTCTGAAGCTTATTGAGACTCACCTGAGAACTAT  HTSHR.SEQ
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FIG. 2

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     TCCCAGTCGTGCATTTTCAAATCTGCCCAATATTTCCAGG   Majority

201  TCCCAGTCGTGCATTTTCAAATCTGCCCAATATTTCCAGG   CAT.SEQ
201  TCCCAGTCGTGCGTTCTCAAATCTGCCCAATATTTCCAGG   COW.SEQ
201  TCCCAGTCGTGCATTTTCAAATCTGCCCAATATTTCCAGG   DOG.SEQ
201  ACCCAGTCTTGCATTTTCGAGTCTGCCCAATATTTCCAGG   MOUSE.SEQ
201  CCCCAGTCGTGCATTTTCAAATCTGCCCAATATTTCCAGG   PTSHR.SEQ
201  TCCCAGTCTTGCCTTTTCGAGCCTGCCCAATATTTCCAGG   RAT.SEQ
201  TCCCAGTCGTGCGTTCTCAAATTTGCCCAATATTTCCAGG   SHEEP.SEQ
201  TCCAAGTCATGCATTTTCTAATCTGCCCAATATTTCCAGA   HTSHR.SEQ

     ATCTACTTGTCAATAGATGCAACTCTGCAGCGGCTGGAAT   Majority

241  ATCTACTTGTCAATAGATGCAACTCTGCAGCGACTGGAAT   CAT.SEQ
241  ATCTACTTGTCAATAGATGCAACTCTGCAGCAGCTGGAAT   COW.SEQ
241  ATCTACTTGTCAATAGATGCAACTCTGCAGCGGCTGGAAT   DOG.SEQ
241  ATCTATTTATCTATAGATGCAACTCTGCAGCGGCTGGAAC   MOUSE.SEQ
241  ATCTACCTGTCAATAGATGCAACTCTACAGCAGCTGGAAT   PTSHR.SEQ
241  ATCTATCTATCCATAGATGCCACTCTGCAGCGACTGGAGC   RAT.SEQ
241  ATCTACTTGTCAATAGATGCGACTTTGCAGCAACTGGAAT   SHEEP.SEQ
241  ATCTACGTATCTATAGATGTGACTCTGCAGCAGCTGGAAT   HTSHR.SEQ

     CACATTCCTTCTACAATTTG                       Majority

281  CACATTCCTTCTACAATTTG                       CAT.SEQ
281  CACATTCCTTCTACAATTTA                       COW.SEQ
281  CACATTCCTTCTACAATTTA                       DOG.SEQ
281  CACATTCTTTCTACAATTTG                       MOUSE.SEQ
281  CACAGTCCTTCTACAATTTG                       PTSHR.SEQ
281  CACATTCTTTCTACAATTTG                       RAT.SEQ
281  CACATTCCTTCTACAATTTA                       SHEEP.SEQ
281  CACACTCCTTCTACAATTTG                       HTSHR.SEQ
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FIG. 2CONT'D

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     TKLDAVYLNKNKYLTAIDKDAFGGVYSGFTLLDVSYTSVT  Majority

200  TKLDAVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVT  HTSHR.PRO
200  TKLDAVYLNKNKYLTVIDKDAFGGVFSGPTLLDVSYTSVT  PTSHR.PRO
200  TKLDAVYLNKNKYLTVIGQDAFAGVYSGPTLLDISYTSVT  BTSHR.PRO
200  TKLDAVYLNKNKYLTAIDQDAFGGVYSGPTLLDVSYTSVT  CTSHR.PRO
200  TKLDAVYLNKNKYLSAIDKDAFGGVYSGPTLLDVSYTSVT  DTSHR.PRO
200  TKLDAVYLNKNKYLTAIDNDAFGGVYSGPTLLDVSSTSVT  MTSHR.PRO
200  TKLDAVYLNKNKYLTAIDKDAFGGVYSGPTLLDVSSTSVT  RTSHR.PRO
200  TKLDAVYLNKNKYLTVIDQDAFAGVYSGPTLLDISYTSVT  STSHRP.PRO

     ALPSKGLEHLKELIARNTWTLKKLPLSPSFLHLTRADLSY  Majority

240  ALPSKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSY  HTSHR.PRO
240  ALPPKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSY  PTSHR.PRO
240  ALPSKGLEHLKELIARNTWTLRKLPLSLSFLHLTRADLSY  BTSHS.PRO
240  ALPSKGLEHLKELIARNTWTLKKLPLTLSFLHLTRADLSY  CTSHR.PRO
240  ALPSKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSY  DTSHR.PRO
240  ALPSKGLEHLKELIAKDTWTLKKLPLSLSFLHLTRADLSY  MTSHR.PRO
240  ALPSKGLEHLKELIAKNTWTLKKLPLSLSFLHLTRADLSY  RTSHS.PRO
240  ALPSKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSY  STSHRP.PRO

     PSHCCAFKNQKKIRGILESLM                     Majority

280  PSHCCAFKNQKKIRGILESLM                     HTSHR.PRO
280  PSHCCAFKNQKKIRGILESLM                     PTSHR.PRO
280  PSHCCAFKNQKKIRGILQSLM                     BTSHR.PRO
280  PSHCCAFKNQKKIRGILESFM                     CTSHR.PRO
280  PSHCCAFKNQKKIRGILESLM                     DTSHR.PRO
280  PSHCCAFKNQKKIRGILESLM                     MTSHR.PRO
280  PSHCCAFKNQKKIRGILESLM                     RTSHR.PRO
280  PSHCCAFKNQKNIRGILQSLM                     STSHRP.PRO
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FIG. 3

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     TCTTACACCAGTGTCACTGCCCTTCCATCCAAAGGCCTGG  Majority

700  TCTTACACCAGTGTCACTGCCCTGCCATCCAAAGGCCTGG  CAT.SEQ
700  TCTTATACCAGTGTCACAGCCCTACCATCCAAAGGCCTGG  COW.SEQ
700  TCTTACACCAGTGTTACTGCCCTGCCATCCAAAGGCCTGG  DOG.SEQ
700  TCTTCCACCAGCGTCACTGCCCTTCCTTCCAAAGGCCTGG  MOUSE.SEQ
700  TCTTATACCAGTGTTACTGCCCTGCCACCCAAAGGCCTGG  PTSHR.SEQ
700  TCTTCCACCAGCGTTACTGCTCTTCCTTCCAAAGGCCTGG  RAT.SEQ
700  TCTTATACCAGTGTCACTGCCCTACCATCCAAAGGCCTGG  SHEEP.SEQ
700  TCTCAAACCAGTGTCACTGCCCTTCCATCCAAAGGCCTGG  HTSHR.SEQ

     AGCACCTGAAGGAACTGATACCAAGAAACACTTGGACTCT  Majority

740  AGCACCTGAAGGAATTGATAGCAAGAAACACTTGGACTCT  CAT.SEQ
740  AACACCTGAAGGAATTGATAGCAAGAAACACTTGGACTCT  COW.SEQ
740  AGCATCTAAAGGAGCTGATAGCAAGAAACACTTGGACTCT  DOG.SEQ
740  AGCACCTCAAAGAACTGATCGCAAAAGACACCTGGACTCT  MOUSE.SEQ
740  AACACCTGAAGGAACTGATAGCAAGAAATACTTGGACTCT  PTSHR.SEQ
740  AGCACCTCAAAGAGCTGATCGCGAAGAACACCTGGACTCT  RAT.SEQ
740  AACACCTGAAGGAATTGATAGCAAGAAACACTTGGACTCT  SHEEP.SEQ
740  AGCACCTGAAGGAACTGATAGCAAGAAACACCTGGACTCT  HTSHR.SEQ

     AAAGAAACTTCCACTTTCCTTGAGTTTCCTTCACCTCACA  Majority

780  AAAGAAACTTCCACTTACCTTGAGTTTCCTTCACCTCACA  CAT.SEQ
780  AAGGAAACTTCCTCTTTCCTTGAGTTTCCTTCACCTCACA  COW.SEQ
780  AAAGAAACTCCCACTTTCCTTGAGTTTCCTTCACCTTACA  DOG.SEQ
780  CAAAAAGCTCCCGCTGTCGTTGAGTTTCCTCCACCTCACT  MOUSE.SEQ
780  AAAGAAACTTCCACTGTCCTTGAGTTTCCTTCACCTCACA  PTSHR.SEQ
780  CAAAAAGCTCCCCCTGTCCTTGAGCTTCCTCCACCTCACT  RAT.SEQ
780  AAAGAAACTTCCTCTTTCCTTGAGTTTCCTTCACCTCACA  SHEEP.SEQ
780  TAAGAAACTTCCACTTTCCTTGAGTTTCCTTCACCTCACA  HTSHR.SEQ

     CGGGCTGACCTTTCTTATCCAAGCCACTGCTGTGCTTTTA  Majority

820  CGGGCTGACCTTTCTTATCCAAGCCACTGCTGTGCTTTTA  CAT.SEQ
820  CGGGCTGACCTTTCTTATCCGAGCCACTGCTGCGCTTTTA  COW.SEQ
820  CGGGCTGACCTTTCTTATCCAAGCCACTGCTGTGCTTTTA  DOG.SEQ
820  CGGGCTGACCTCTCTTACCCGAGCCACTGCTGCGCTTTTA  MOUSE.SEQ
820  CGAGCTGACCTTTCTTATCCAAGCCACTGCTGTGCTTTTA  PTSHR.SEQ
820  CGGGCTGACCTCTCTTACCCAAGTCACTGCTGTGCTTTTA  RAT.SEQ
820  CGGGCTGACCTTTCTTATCCGAGCCACTGCTGTGCTTTTA  SHEEP.SEQ
820  CGGGCTGACCTTTCTTACCCAAGCCACTGCTGTGCCTTTA  HTSHR.SEQ

     AGAATCAGAAGAAAATCAGACCAATCCTTGACTCTTTAAT  Majority

860  AGAATCAGAAGAAAATCAGAGGAATCCTTGAGTCCTTCAT  CAT.SEQ
860  AGAATCAGAAGAAAATCAGAGGAATCCTTCAGTCTTTAAT  COW.SEQ
860  AGAATCAGAAGAAAATCAGAGGAATCCTTGAGTCCTTAAT  DOG.SEQ
860  AGAACCAGAAGAAAATCAGGGGAATCCTGGAGTCTTTGAT  MOUSE.SEQ
860  AGAATCAGAAGAAGATCAGAGGAATCCTTGAGTCTTTAAT  PTSHR.SEQ
860  AGAACCAGAAGAAAATCAGGGGAATCCTAGAGTCTTTGAT  RAT.SEQ
860  AGAATCAGAAGAATATCAGAGGAATCCTTCAGTCTTTAAT  SHEEP.SEQ
860  AGAATCAGAAGAAAATCAGAGGAATCCTTGAGTCCTTGAT  HTSHR.SEQ
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FIG. 4

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     KELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  Majority

250  KELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  HTSHR.PRO
250  KELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  PTSHR.PRO
250  KELIARNTWTLRKLPLSLSFLHLTRADLSYPSHCCAFKNQ  BTSHR.PRO
250  KELIARNTWTLKKLPLTLSFLHLTRADLSYPSHCCAFKNQ  CTSHR.PRO
250  KELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  DTSHR.PRO
250  KELIAKDTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  MTSHR.PRO
250  KELIAKNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  RTSHR.PRO
250  KELIARNTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQ  STSHRP.PRO

     KKIRGILESLMCNESSIRSLRQRKSVNALNGPFYQEYEED  Majority

290  KKIRGILESLMCNESSMQSLRQRKSVNALNSPLHQEYEEN  HTSHR.PRO
290  KKIRGILESLMCNESSIRSLRQRKSVNAVNGPFYQEYEED  PTSHR.PRO
290  KKIRGILQSLMCNESSIRGLRQRKSASALNGPFYQEYEDX  BTSHS.PRO
290  KKIRGILESFMCNDSSIRSLRQRKSVNALNGPFDQEYEEY  CTSHR.PRO
290  KKIRGILESLMCNESSIRSLRQRKSVNTLNGPFDQEYEEY  DTSHR.PRO
290  KKIRGILESLMCNESSIRNLRQRKSVNILRGPIYQEYEED  MTSHR.PRO
290  KKIRGILESLMCNESSIRNLRQRKSVNVMRGPVYQEYEEG  RTSHS.PRO
290  KNIRGILQSLMCNESSIWGLRQRKSASALNGPFYQEYEED  STSHRP.PRO

     LDGSSAGYKENSKFQDTHSNSHYYVFFEEQEDEIIGFGQE  Majority

330  LGDSIVGYKEKSKFQDTHNNAHYYVFFEEQEDEIIGFGQE  HTSHR.PRO
330  LGDTSVGNKENSKFQDTHSNSHYYVFFEEQEDEIIGFGQE  PTSHR.PRO
330  LGDGSAGYKENSKFQDTQSNSHYYVFFEEQEDEIIGFGQQ  BTSHR.PRO
330  LGDSHAGYKDNSKFQDTRSNSHYYVFFEEQXDEILGFGQE  CTSHR.PRO
330  LGDSHAGYKDNSQFQDTDSNSHYYVFFEEQEDEILGFGQE  DTSHR.PRO
330  PGDNSVGYKQNSKFQESPSNSHYYVFFEEQEDEVVGFGQE  MTSHR.PRO
330  LGDNHVGYKQNSKFQEGPSNSHYYVFFEEQEDEIIGFGQE  RTSHR.PRO
330  LGDGSAGYKENSKFQDTHSNSHYYVFFEDQEDEIIGFGQE  STSHRP.PRO

     LKNPQEETLQAFDSHYDYTVCGGSEDMVCTPKSDEFNPCE  Majority

370  LKNPQEETLQAFDSHYDYTICGDSEDMVCTPKSDEFNPCE  HTSHR.PRO
370  LKNPQEETLQAFDSHYDYTVCGGSEDMVCTPKSDEFNPCE  PTSHR.PRO
370  LKNPQEETLQAFDSHYDYTVCGGSEDMVCTPKSDEFNPCE  BTSHR.PRO
370  LKNPQEETLQAFDSHYDYTVCGGNEDMVCTPKSDEFNPCE  CTSHR.PRO
370  LKNPQEETLQAFDSHYDYTVCGGNEDMVCTPKSDEFNPCE  DTSHR.PRO
370  LKNPQEETLQAFESHYDYTVCGDNEDMVCTPKSDEFNPCE  MTSHR.PRO
370  LKNPQEETLQAFDSHYDYTVCGDNEDMVCTPKSDEFNPCE  RTSHR.PRO
370  LKNPQEETLQAFDNHYDYTVCGGSEEMVCTPKSDEFNPCE  STSHRP.PRO

     DIMGYKFLRIVVWFVSLLALLGNVFVLVILLTSHYKLTVP  Majority

410  DIMGYKFLRIVVWFVSLLALLGNVFVLLILLTSHYKLNVP  HTSHR.PRO
410  DIMGYRFLRIVVWFVSLLALLGNVFVLVILLTSHYKLTVP  PTSHR.PRO
410  DIMGYKFLRIVVWFVSLLALLGNVFVLVILLTSHYKLTVP  BTSHR.PRO
410  DIMGYKFLRIVVWFVSLLALLGNVFVLIILLTSHYKLTVP  CTSHR.PRO
410  DIMGYKFLRIVVWFVSLLALLGNVFVLIVLLTSHYKLTVP  DTSHR.PRO
410  DIMGYRFLRIVVWFVSLLALLGNIFVLLILLTSHYKLTVP  MTSHR.PRO
410  DIMGYKFLRIVVWFVSPMALLGNVFVLFVLLTSHYKLTVP  RTSHR.PRO
410  DIMGYKFLRIVVWFVSLLALLGNVFVLVILLTSHYKLTVP  STSHRP.PRO
```

FIG. 5

```
    GGAACTGATAGCAAGAAACACTTGGACTCTAAAGAAACTT  Majority

750 GGAATTGATAGCAAGAAACACTTGGACTCTAAAGAAACTT  CAT.SEQ
750 GGAATTGATAGCAAGAAACACTTGGACTCTAAGGAAACTT  COW.SEQ
750 GGAGCTGATAGCAAGAAACACTTGGACTCTAAAGAAACTC  DOG.SEQ
750 AGAACTGATCGCAAAAGACACCTGGACTCTCAAAAAGCTC  MOUSE.SEQ
750 GGAACTGATAGCAAGAAATACTTGGACTCTAAAGAAACTT  PTSHR.SEQ
750 AGAGCTGATCGCGAAGAACACCTGGACTCTCAAAAAGCTC  RAT.SEQ
750 GGAATTGATAGCAAGAAACACTTGGACTCTAAAGAAACTT  SHEEP.SEQ
750 GGAACTGATAGCAAGAAACACCTGGACTCTTAAGAAACTT  HTSHR.SEQ

    CCACTTTCCTTGAGTTTCCTTCACCTCACACGGGCTGACC  Majority

790 CCACTTACCTTGAGTTTCCTTCACCTCACACGGGCTGACC  CAT.SEQ
790 CCTCTTTCCTTGAGTTTCCTTCACCTCACACGGGCTGACC  COW.SEQ
790 CCACTTTCCTTGAGTTTCCTTCACCTTACACGGGCTGACC  DOG.SEQ
790 CCGCTGTCGTTGAGTTTCCTCCACCTCACTCGGGCTGACC  MOUSE.SEQ
790 CCACTGTCCTTGAGTTTCCTTCACCTCACACGAGCTGACC  PTSHR.SEQ
790 CCCCTGTCCTTGAGCTTCCTCCACCTCACTCGGGCTGACC  RAT.SEQ
790 CCTCTTTCCTTGAGTTTCCTTCACCTCACACGGGCTGACC  SHEEP.SEQ
790 CCACTTTCCTTGAGTTTCCTTCACCTCACACGGGCTGACC  HTSHR.SEQ

    TTTCTTATCCAAGCCACTGCTGTGCTTTTAAGAATCAGAA  Majority

830 TTTCTTATCCAAGCCACTGCTGTGCTTTTAAGAATCAGAA  CAT.SEQ
830 TTTCTTATCCGAGCCACTGCTGCGCTTTTAAGAATCAGAA  COW.SEQ
830 TTTCTTATCCAAGCCACTGCTGTGCTTTTAAGAATCAGAA  DOG.SEQ
830 TCTCTTACCCGAGCCACTGCTGCGCTTTTAAGAACCAGAA  MOUSE.SEQ
830 TTTCTTATCCAAGCCACTGCTGTGCTTTTAAGAATCAGAA  PTSHR.SEQ
830 TCTCTTACCCAAGTCACTGCTGTGCTTTTAAGAACCAGAA  RAT.SEQ
830 TTTCTTATCCGAGCCACTGCTGTGCTTTTAAGAATCAGAA  SHEEP.SEQ
830 TTTCTTACCCAAGCCACTGCTGTGCCTTTAAGAATCAGAA  HTSHR.SEQ

    GAAAATCAGAGGAATCCTTGAGTCTTTAATGTGTAATGAG  Majority

870 GAAAATCAGAGGAATCCTTGAGTCCTTCATGTGTAATGAC  CAT.SEQ
870 GAAAATCAGAGGAATCCTTCAGTCTTTAATGTGTAACGAG  COW.SEQ
870 GAAAATCAGAGGAATCCTTGAGTCCTTAATGTGTAATGAA  DOG.SEQ
870 GAAAATCAGGGGAATCCTGGAGTCTTTGATGTGTAATGAG  MOUSE.SEQ
870 GAAGATCAGAGGAATCCTTGAGTCTTTAATGTGTAATGAG  PTSHR.SEQ
870 GAAAATCAGGGGAATCCTAGAGTCTTTGATGTGTAATGAG  RAT.SEQ
870 GAATATCAGAGGAATCCTTCAGTCTTTAATGTGTAACGAG  SHEEP.SEQ
870 GAAAATCAGAGGAATCCTTGAGTCCTTGATGTGTAATGAG  HTSHR.SEQ

    AGCAGTATTCGGAGCCTGCGTCAGAGAAAATCTGTGAATG  Majority

910 AGCAGTATTCGGAGCCTGCGTCAGAGAAAATCTGTGAATG  CAT.SEQ
910 AGCAGTATTCGGGGCCTGCGTCAGAGAAAATCCGCAAGTG  COW.SEQ
910 AGCAGTATTCGGAGCCTGCGCCAGAGAAAATCTGTGAATA  DOG.SEQ
910 AGCAGTATCCGGAACCTTCGTCAAAGGAAATCAGTGAACA  MOUSE.SEQ
910 AGCAGTATTCGGAGCCTGCGTCAGAGAAAATCTGTGAATG  PTSHR.SEQ
910 AGTAGTATCCGGAACCTGCGTCAAAGAAAGTCAGTGAACG  RAT.SEQ
910 AGCAGTATTTGGGGCCTGCGTCAGAGAAAATCCGCGAGTG  SHEEP.SEQ
910 AGCAGTATGCAGAGCTTGCGCCAGAGAAAATCTGTGAATG  HTSHR.SEQ
```

FIG. 6

```
     CTTTGAATGGTCCCTTCTACCAGGAATATGAAGAGGATCT  Majority

 950 CTTTGAATGGTCCCTTCGACCAGGAATATGAAGAGTATCT  CAT.SEQ
 950 CTTTGAATGGTCCCTTCTACCAGGAATATGAGGATNNNCT  COW.SEQ
 950 CTTTGAATGGCCCCTTTGACCAGGAATATGAAGAGTATCT  DOG.SEQ
 950 TCTTGAGGGGTCCCATCTACCAGGAATATGAAGAAGATCC  MOUSE.SEQ
 950 CTGTAAATGGTCCCTTTTACCAAGAATATGAAGAGGATCT  PTSHR.SEQ
 950 TCATGAGGGGTCCCGTCTACCAGGAATATGAAGAAGGTCT  RAT.SEQ
 950 CTTTGAATGGTCCCTTCTACCAGGAATATGAAGAGGATCT  SHEEP.SEQ
 950 CCTTGAATAGCCCCCTCCACCAGGAATATGAAGAGAATCT  HTSHR.SEQ

     GGGTGACAGCAGTGTTGGGTACAAGGAAAACTCCAAGTTC  Majority

 990 AGGTGACAGCCATGCTGGATATAAGGACAACTCTAAGTTC  CAT.SEQ
 990 GGGTGATGGCAGTGCTGGGTACAAGGAGAACTCCAAGTTC  COW.SEQ
 990 GGGTGACAGCCATGCTGGGTACAAGGACAACTCTCAGTTC  DOG.SEQ
 990 GGGTGACAACAGTGTTGGGTACAAACAAAACTCCAAGTTC  MOUSE.SEQ
 990 GGGCGACACGAGTGTTGGGAATAAGGAAAACTCCAAGTTC  PTSHR.SEQ
 990 GGGTGACAACCATGTTGGGTACAAACAAAACTCCAAGTTC  RAT.SEQ
 990 GGGTGATGGCAGTGCTGGGTACAAGGAGAACTCCAAGTTC  SHEEP.SEQ
 990 GGGTGACAGCATTGTTGGGTACAAGGAAAAGTCCAAGTTC  HTSHR.SEQ

     CAGGATACCCATAGCAACTCTCATTATTATGTCTTCTTTG  Majority

1030 CAGGATACTCGCAGCAACTCTCATTATTATGTCTTCTTTG  CAT.SEQ
1030 CAAGATACCCAAAGCAACTCTCATTACTATGTCTTCTTTG  COW.SEQ
1030 CAGGATACCGATAGCAATTCTCATTATTATGTCTTCTTCG  DOG.SEQ
1030 CAGGAGAGCCCAAGCAACTCTCACTATTACGTCTTCTTTG  MOUSE.SEQ
1030 CAGGATACCCATAGCAACTCCCATTACTACGTCTTCTTTG  PTSHR.SEQ
1030 CAGGAGGGCCCAAGCAACTCTCACTATTACGTCTTCTTTG  RAT.SEQ
1030 CAAGATACCCACAGCAACTCTCATTACTATGTCTTCTTTG  SHEEP.SEQ
1030 CAGGATACTCATAACAACGCTCATTATTACGTCTTCTTTG  HTSHR.SEQ

     AAGAACAAGAGGATGAGATCATTGGTTTTGG            Majority

1070 AAGAACAANNNGACGAGATCCTTGGTTTTGG           CAT.SEQ
1070 AGGAGCAAGAAGATGAGATCATCGGTTTTGG          COW.SEQ
1070 AAGAACAAGAAGATGAGATCCTCGGTTTTGG          DOG.SEQ
1070 AAGAACAAGAGGATGAGGTCGTTGGTTTCGG          MOUSE.SEQ
1070 AAGAACAAGAGGATGAGATCATTGGTTTTGG          PTSHR.SEQ
1070 AAGAACAAGAGGACGAGATCATCGGTTTCGG          RAT.SEQ
1070 AGGATCAAGAAGATGAGATCATCGGTTTTGG          SHEEP.SEQ
1070 AAGAACAAGAGGATGAGATCATTGGTTTTGG          HTSHR.SEQ
```

FIG. 6CONT'D

```
    SHYYVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTV  Majority

750 AHYYVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTI  CAT.SEQ
750 SHYYVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTV  COW.SEQ
750 SHYYVFFEEQEDEIIGFGQQLKNPQEETLQAFDSHYDYTV  DOG.SEQ
750 SHYYVFFEEQXDEILGFGQELKNPQEETLQAFDSHYDYTV  MOUSE.SEQ
750 SHYYVFFEEQEDEILGFGQELKNPQEETLQAFDSHYDYTV  PTSHR.SEQ
750 SHYYVFFEEQEDEVVGFGQELKNPQEETLQAFESHYDYTV  RAT.SEQ
750 SHYYVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTV  SHEEP.SEQ
750 SHYYVFFEDQEDEIIGFGQELKNPQEETLQAFDNHYDYTV  HTSHR.SEQ

    CGGSEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  Majority

790 CGDSEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  CAT.SEQ
790 CGGSEDMVCTPKSDEFNPCEDIMGYRFLRIVVWFVSLLAL  COW.SEQ
790 CGGSEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  DOG.SEQ
790 CGGNEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  MOUSE.SEQ
790 CGGNEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  PTSHR.SEQ
790 CGDNEDMVCTPKSDEFNPCEDIMGYRFLRIVVWFVSLLAL  RAT.SEQ
790 CGDNEDMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSPMAL  SHEEP.SEQ
790 CGGSEEMVCTPKSDEFNPCEDIMGYKFLRIVVWFVSLLAL  HTSHR.SEQ

    LGNVFVLVILLTSHYKLTVPRFLMCNLAFADFCMGMYLLL  Majority

830 LGNVFVLLILLTSHYKLNVPRFLMCNLAFADFCMGMYLLL  CAT.SEQ
830 LGNVFVLVILLTSHYKLTVPRFLMCNLAFADFCMGMYLLL  COW.SEQ
830 LGNVFVLVILLTSHYKLTVPRFLMCNLAFADFCMGLYLLL  DOG.SEQ
830 LGNVFVLIILLTSHYKLTVPRFLMCNLAFADFCMGMYLLL  MOUSE.SEQ
830 LGNVFVLIVLLTSHYKLTVPRFLMCNLAFADFCMGMYLLL  PTSHR.SEQ
830 LGNIFVLLILLTSHYKLTVPRFLMCNLAFADFCMGVYLLL  RAT.SEQ
830 LGNVFVLFVLLTSHYKLTVPRFLMCNLAFADFCMGVYLLL  SHEEP.SEQ
830 LGNVFVLVILLTSHYKLTVPRFLMCNLAFADFCMGLYLLL  HTSHR.SEQ

    IASVDLYTHSEYYNHAIDWQTGPGCNTAGFF           Majority

870 IASVDLYTHSEYYNHAIDWQTGPGCNTAGFF           CAT.SEQ
870 IASVDLYTQSEYYNHAIDWQTGPGCNTAGFF           COW.SEQ
870 IASVDLYTQSEYYNHAIDWQTGPGCNTAGFF           DOG.SEQ
870 IASVDLYTHSEYYNHAIDWQTGPGCNAAGFF           MOUSE.SEQ
870 IASVDLYTHSEYYNHAIDWQTGPGCNTAGFF           PTSHR.SEQ
870 IASVDLYTHSEYYNHAIDWQTGPGCNTAGFF           RAT.SEQ
870 IASVDLYTHTEYYNHAIDWQTGPGCNTAGFF           SHEEP.SEQ
870 IASVDLYTQSEYYNHAIDWQTGPGCNTAGFF           HTSHR.SEQ
```

FIG. 7

```
    GCCAAGAGCTCAAAAACCCCCAGGAAGAGACCCTCCAGGC  Majority

700 GCCAGGAGCTTAAAAACCCACAAGAAGAGACCCTACAGGC  CAT.SEQ
700 GCCAACAGCTCAAAAACCCCCAGGAGGAGACCCTGCAGGC  COW.SEQ
700 GGCAGGAGCTTAAAAACCCACAGGAAGAGACCCTCCAGGC  DOG.SEQ
700 GCCAAGAGCTCAAAAATCCTCAGGAAGAGACTCTCCAAGC  MOUSE.SEQ
700 GCCAAGAGCTCAAAAACCCCCAGGAAGAGACCCTCCAGGC  PTSHR.SEQ
700 GCCAAGAGCTCAAAAATCCTCAGGAAGAGACTCTCCAAGC  RAT.SEQ
700 GCCAAGAGCTTAAAAACCCCCAGGAGGAGACCCTGCAGGC  SHEEP.SEQ
700 GCCAGGAGCTCAAAAACCCCCAGGAAGAGACTCTACAAGC  HTSHR.SEQ

    CTTTGACAGCCATTATGACTACACCGTGTGTCGGGGCAGT  Majority

740 CTTCGATAGCCATTATGACTACACTGTGTGTGGAGGCAAT  CAT.SEQ
740 CTTTGACAGCCATTACGACTATACCGTGTGTGGGGGCAGT  COW.SEQ
740 CTTTGATAGCCATTATGACTACACTGTGTGTGGTGGCAAT  DOG.SEQ
740 CTTCGAGAGCCACTATGACTACACGGTGTGTGGGGACAAC  MOUSE.SEQ
740 CTTTGACAGCCATTACGACTACACCGTGTGTGGGGGCAGT  PTSHR.SEQ
740 CTTCGACAGCCACTATGACTACACTGTGTGTGGGGACAAC  RAT.SEQ
740 CTTTGACAACCATTACGACTATACCGTGTGCGGGGGGAGT  SHEEP.SEQ
740 TTTTGACAGCCATTATGACTACACCATATGTGGGGACAGT  HTSHR.SEQ

    GAGGACATGGTGTGTACCCCCAAGTCAGATCAGTTCAACC  Majority

780 GAAGACATGGTGTGTACTCCCAAGTCAGATGAGTTCAACC  CAT.SEQ
780 GAGGACATGGTGTGTACCCCCAAGTCGGATGAGTTCAACC  COW.SEQ
780 GAAGACATGGTGTGTACTCCTAAGTCAGATGAGTTCAACC  DOG.SEQ
780 GAGGACATGGTGTGTACCCCCAAGTCGGACGAGTTTAACC  MOUSE.SEQ
780 GAAGACATGGTGTGCACCCCCAAGTCAGATGAGTTCAACC  PTSHR.SEQ
780 GAGGACATGGTGTGTACCCCCAAGTCAGACGAGTTTAACC  RAT.SEQ
780 GAGGAGATGGTGTGTACCCCCAAGTCGGATGAGTTCAACC  SHEEP.SEQ
780 GAAGACATGGTGTGTACCCCCAAGTCCGATGAGTTCAACC  HTSHR.SEQ

    CCTGTGAAGACATCATGGGCTACAAGTTCCTGAGAATTGT  Majority

820 CCTGTGAAGACATAATGGGCTACAAGTTCCTGAGAATTGT  CAT.SEQ
820 CCTGTGAGGACATCATGGGCTACAAGTTCCTGAGAATCGT  COW.SEQ
820 CCTGTGAAGACATAATGGGCTACAAGTTCCTGAGGATTGT  DOG.SEQ
820 CCTGTGAAGATATCATGGGCTACAGGTTCCTGAGAATCGT  MOUSE.SEQ
820 CCTGTGAAGACATAATGGGCTACAGGTTCCTGAGAATCGT  PTSHR.SEQ
820 CCTGTGAAGATATCATGGGCTACAAGTTCCTGAGAATCGT  RAT.SEQ
820 CCTGTGAGGACATCATGGGCTACAAGTTCCTGAGAATTGT  SHEEP.SEQ
820 CGTGTGAAGACATAATGGGCTACAAGTTCCTGAGAATTGT  HTSHR.SEQ

    GGTGTGGTTTGTTAGTCTGCTGGCTCTCCTGGGCAATGTC  Majority

860 GGTGTGGTTTGTTAGTCTGCTGGCTCTCCTGGGCAATGTC  CAT.SEQ
860 GGTGTGGTTTGTGAGTCTGCTGGCTCTCCTGGGCAACGTC  COW.SEQ
860 GGTGTGGTTTGTTAGTCTGCTGGCTCTCCTGGGCAATGTC  DOG.SEQ
860 GGTGTGGTTTGTCAGTCTGCTGGCTCTCCTGGGCAATATC  MOUSE.SEQ
860 GGTGTGGTTCGTTAGCCTGCTGGCTCTCCTGGGCAATGTC  PTSHR.SEQ
860 GGTATGGTTTGTCAGTCCGATGGCTCTCCTGGGCAACGTC  RAT.SEQ
860 GGTGTGGTTTGTGAGTCTGCTGGCTCTCCTGGGCAACGTC  SHEEP.SEQ
860 GGTGTGGTTCGTTAGTCTGCTGGCTCTCCTGGGCAATGTC  HTSHR.SEQ
```

FIG. 8

4D7 - HC

DVQLKHSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGL
INPYTGGTNYNQKFKGKAKLTVDKSSSTAFMELLSLTSEDSAVYYCARDG
NLDYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA
HPASKTKVD

FIG. 9

4D7 - HC

DVQLKHSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGL 50
PCR primer
CDR I

INPYTGGTNYNQKFKGKAKLTVDKSSSTAFMELLSLTSEDSAVYYCARDG 100
CDR II
CDR III

NLDYWGQGTTLTVSS**AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE** 150
constant region

PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA 200

HPASKTKVD 209
PCR primer

FIG. 10

4D7 - LC

SIVMSQSPASLAVSLGQRATISCRASETVDNYGFSFMHWFQQIPGQPPKL
LIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPY
TFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV
KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA
THKTSTSPIVKSFNRNEC

FIG. 11

4D7 - LC

SIVMSQSPASLAVSLGQRATISCRASETVDNYGFSFMHWFQQIPGQPPKL 50
PCR primer CDR I

LIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPY 100
CDR II CDR III

TFGGGTKLEIK**RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV** 150
constant region

KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA 200

THKTSTSPIVKSFNRNEC 218
PCR primer

FIG. 12

16E5 - HC

DVQLVQSGPELVKPGASVKMSCKASGYSFTGYNMHWVKQSHGKSLEWIGY
IDPYNGATSYNQKFEDKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARRW
DWDPYAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTSGSSVTLGCLVK
GYFPEPVTLTWNSGSLSSGVHTSPAVLQSDLYTLSSSVTVTSSTWPSQSI
TCNVAHPASKTKVD

FIG. 13

16E5 - HC

DVQLVQSGPELVKPGASVKMSCKASGYSFTGYNMHWVKQSHGKSLEWIGY 50
PCR primer CDR I

IDPYNGATSYNQKFEDKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARRW 100
CDR II

DWDPYAMDYWGQGTSVTVSS**AKTTAPSVYPLAPVCGDTSGSSVTLGCLVK** 150
CDR III constant region

GYFPEPVTLTWNSGSLSSGVHTSPAVLQSDLYTLSSSVTVTSSTWPSQSI 200

TCNVAHPASKTKVD 214
PCR primer

FIG. 14

16E5 - LC

DILLTQSPAILSVSPGERVSFSCRASQSIGTSIHWYQQRTNGSPRLLIKY
ASESISGIFSRFSGSGSGTDFTLTINSVESEDIADYYCQQSNRWPLTFGA
GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI
DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT
STSPIVKSFNRNEC

FIG. 15

16E5 - LC

DILLTQSPAILSVSPGERVSFSCRASQSIGTSIHWYQQRTNGSPRLLIKY 50
PCR primer CDR I

ASESISGIFSRFSGSGSGTDFTLTINSVESEDIADYYCQQSNRWPLTFGA 100
CDR II CDR III

GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI 150
constant region

DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT 200

STSPIVKSFNRNEC 214
PCR primer

FIG. 16

17D2 - HC

DVQIQQSGPELVKPGASVKMSCKASGYSFTAYNMHWVKQTHGKSLEWIGY
IDPYSGATSYHQKFKGKATLTVDKSSSTAYMRLNSLTSEDSAVYYCARRW
DWDPYAMDYWGQGTSVTVSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVK
GYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSAWPSQTV
TCSVAHPASNTTVD

FIG. 17

17D2 - HC

DVQIQQSGPELVKPGASVKMSCKASGYSFTAYNMHWVKQTHGKSLEWIGY 50
PCR primer CDR I

IDPYSGATSYHQKFKGKATLTVDKSSSTAYMRLNSLTSEDSAVYYCARRW 100
CDR II

DWDPYAMDYWGQGTSVTVSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVK 150
CDR III constant region

GYFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSAWPSQTV 200

214
TCSVAHPASNTTVD
PCR primer

FIG. 18

17D2 - LC

SVEMSQSPAILSVSPGERISFSCRASQSIGTSIHWYQQRTNGSPRLLIKY
ASASISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQSNSWPLTFGA
GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI
DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT
STSPIVKSFNRNEC

FIG. 19

17D2 - LC

SVEMSQSPAILSVSPGERISFSCRASQSIGTSIHWYQQRTNGSPRLLIKY 50
PCR primer CDR I

ASASISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQSNSWPLTFGA 100
CDR II CDR III

GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI 150
constant region

DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT 200

STSPIVKSFNRNEC 214
PCR primer

FIG. 20

14D3 - HC

DVQMQQPGPELVKPGASLKMSCKASGYSFTGYNMHWVKQSHGKSLEWIGY
IDPYSGATSYNQKFEGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARRW
DWDPYAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTSGSSVTLGCLVK
GYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI
TCNVAHPASNTKVD

FIG. 21

14D3 - HC

DVQMQQPGPELVKPGASLKMSCKASGYSFTGYNMHWVKQSHGKSLEWIGY 50
PCR primer CDR I

IDPYSGATSYNQKFEGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARRW 100
CDR II

DWDPYAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTSGSSVTLGCLVK 150
CDR III constant region

GYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI 200

214
TCNVAHPASNTKVD
PCR primer

FIG. 22

14D3 - LC

NILMTQSPAILSVSPGERVSFACRASQSIGTSIHWYQQRTNGSPRLLIKY
ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQTNRWPLTFGA
GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI
DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT
STSPIVKSFNRNEC

FIG. 23

14D3 - LC

NILMTQSPAILSVSPGERVSFACRASQSIGTSIHWYQQRTNGSPRLLIKY 50
PCR primer CDR I

ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQTNRWPLTFGA 100
CDR II CDR III

GTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI 150
constant region

DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT 200

STSPIVKSFNRNEC 214
PCR primer

FIG. 24

4D7 - HC

gacgtccagctgaagcactcaggacctgagctggtgaagcctggagcttc
aatgaagatatcctgtaaggcttctggttactcattcactggctacacca
tgaactgggtgaagcagagccatggaaagaaccttgagtggattggactt
attaatccttacactggtggtactaactacaaccagaagttcaagggcaa
ggccaaattaactgtagacaagtcatccagcacagccttcatggagctcc
tcagtctgacatctgaggactctgcagtctattactgtgcaagagatggt
aaccttgactactggggccaaggcaccactctcacagtctcctcagccaa
aacgacaccccatctgtctatccactggcccctggatctgctgcccaaa
ctaactccatggtgaccctgggatgcctggtcaagggctatttccctgag
ccagtgacagtgacctggaactctggatccctgtccagcggtgtgcacac
cttcccagctgtcctgcagtctgacctctacactctgagcagctcagtga
ctgtcccctccagcacctggcccagcgagaccgtcacctgcaacgttgcc
cacccagccagcaagaccaaggtcgac

FIG. 25

4D7 - HC

gacgtccagctgaagcactcaggacctgagctggtgaagcctggagcttc 50
PCR primer

aatgaagatatcctgtaaggcttctggttactcattcactggctacacca 100
CDR I

tgaactgggtgaagcagagccatggaaagaaccttgagtggattggactt 150

attaatccttacactggtggtactaactacaaccagaagttcaagggcaa 200
CDR II

ggccaaattaactgtagacaagtcatccagcacagccttcatggagctcc 250

tcagtctgacatctgaggactctgcagtctattactgtgcaagagatggt 300
CDR III

aaccttgactactggggccaaggcaccactctcacagtctcctcagccaa 350

aacgacaccccatctgtctatccactggcccctggatctgctgcccaaa 400
constant region

ctaactccatggtgaccctgggatgcctggtcaagggctatttccctgag 450

ccagtgacagtgacctggaactctggatccctgtccagcggtgtgcacac 500

cttcccagctgtcctgcagtctgacctctacactctgagcagctcagtga 550

ctgtcccctccagcacctggcccagcgagaccgtcacctgcaacgttgcc 600

cacccagccagcaagaccaaggtcgac 627
PCR primer

FIG. 26

4D7 - LC

agcattgtgatgtcacagtcgccagcttctttggctgtgtctctagggca
gagggccaccatctcctgcagagccagcgaaactgttgataattatggct
ttagttttatgcactggttccaacagataccgggacagccacccaaactc
ctcatctatgctgcatccaaccaaggatccggggtccctgccaggtttag
tggcagtgggtctgggacagacttcagcctcaacatccatcctatggagg
aggatgatactgcaatgtatttctgtcagcaaagtaaggaggttccgtac
acgttcggaggggggaccaagctggaaataaaacgggctgatgctgcacc
aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg
cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc
aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg
gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca
cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc
actcacaagacatcaacttcacccattgtcaagagcttcaacaggaatga
gtgt

FIG. 27

4D7 - LC

agcattgtgatgtcacagtcgccagcttctttggctgtgtctctagggca 50
PCR primer

gagggccaccatctcctgcagagccagcgaaactgttgataattatggct 100
CDR I

ttagttttatgcactggttccaacagataccgggacagccacccaaactc 150

ctcatctatgctgcatccaaccaaggatccggggtccctgccaggtttag 200
CDR II

tggcagtgggtctgggacagacttcagcctcaacatccatcctatggagg 250

aggatgatactgcaatgtatttctgtcagcaaagtaaggaggttccgtac 300
CDR III

acgttcggaggggggaccaagctggaaataaaacgggctgatgctgcacc 350
constant region

aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg 400

cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc 450

aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg 500

gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca 550

cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc 600

actcacaagacatcaacttcacccattgtcaagagcttcaacaggaatga 650
PCR primer

gtgt 654

FIG. 28

16E5 - HC

gacgtccagttggtgcaatctggacctgagctggtgaagcctggagcttc
agtgaagatgtcctgcaaggcttctggttactcattcactggctacaaca
tgcactgggtgaagcagagccatggaaagagccttgagtggattgggtat
attgatccttacaatggtgctactagctacaaccagaaattcgaggacaa
ggccacattgactgtagacaaatcttccagcacagcctacatgcagctca
acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg
gactgggacccttatgctatggactactggggtcaaggaacctcagtcac
cgtctcctcagccaaaacaacagccccatcggtctatccactggcccctg
tgtgtggagatacaagtggctcctcggtgactctaggatgcctggtcaag
ggttatttccctgagccagtgaccttgacctggaactctggatccctgtc
cagtggtgtgcacacctccccagctgtcctgcagtctgacctctacaccc
tcagcagctcagtgactgtaacctcgagcacctggcccagccagtccatc
acctgcaatgtggcccacccggccagcaagaccaaggtcgac

FIG. 29

16E5 - HC

gacgtccagttggtgcaatctggacctgagctggtgaagcctggagcttc 50
PCR primer

agtgaagatgtcctgcaaggcttctggttactcattcactggctacaaca 100
CDR I

tgcactgggtgaagcagagccatggaaagagccttgagtggattgggtat 150

attgatccttacaatggtgctactagctacaaccagaaattcgaggacaa 200
CDR II

ggccacattgactgtagacaaatcttccagcacagcctacatgcagctca 250

acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg 300
CDR III

gactgggacccttatgctatggactactggggtcaaggaacctcagtcac 350

cgtctcctcagccaaaacaacagccccatcggtctatccactggcccctg 400
constant region

tgtgtggagatacaagtggctcctcggtgactctaggatgcctggtcaag 450

ggttatttccctgagccagtgaccttgacctggaactctggatccctgtc 500

cagtggtgtgcacacctccccagctgtcctgcagtctgacctctacaccc 550

tcagcagctcagtgactgtaacctcgagcacctggcccagccagtccatc 600

acctgcaatgtggcccacccggccagcaagaccaaggtcgac 642
PCR primer

FIG. 30

16E5 - LC

gacatcttgctgactcagtctccagccatcctgtctgtgagtccaggaga
aagagtcagtttctcctgcagggccagtcagagcattggcacaagcatac
actggtatcagcaaagaacaaatggttctccaaggcttctcataaagtat
gcttctgagtccatctctgggatattttctaggtttagtggcagtggatc
agggacagattttactcttaccatcaacagtgtggagtctgaagatattg
cagattattactgtcaacaaagtaataggtggccgctcacgttcggagct
gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat
cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt
gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt
gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga
cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg
acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca
tcaacttcacccattgtcaagagcttcaacaggaatgagtgt

FIG. 31

16E5 - LC

gacatcttgctgactcagtctccagccatcctgtctgtgagtccaggaga 50

PCR primer

aagagtcagtttctcctgcagggccagtcagagcattggcacaagcatac 100

CDR I

actggtatcagcaaagaacaaatggttctccaaggcttctcataaagtat 150

gcttctgagtccatctctgggatattttctaggtttagtggcagtggatc 200

CDR II

agggacagattttactcttaccatcaacagtgtggagtctgaagatattg 250

cagattattactgtcaacaaagtaataggtggccgctcacgttcggagct 300

CDR III

gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat 350

constant region

cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt 400

gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt 450

gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga 500

cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg 550

acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca 600

tcaacttcacccattgtcaagagcttcaacaggaatgagtgt 642

PCR primer

FIG. 32

17D2 - HC

gacgtccagatccagcagtctgggcctgagctggtgaagcctggagcttc
agtgaagatgtcctgcaaggcttctggttactcattcactgcctacaaca
tgcactgggtgaagcagacccatggaaagagccttgagtggattggttat
attgatccttacagtggtgctactagctaccaccagaaattcaagggcaa
ggccacattgactgttgacaaatcttccagcacagcctacatgcgcctca
acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg
gactgggacccttatgctatggactactggggtcaaggaacctcagtcac
cgtctcctcagccaaaacaacacccccatcagtctatccactggcccctg
ggtgtggagatacaactggttcctccgtgactctgggatgcctggtcaag
ggctacttccctgagtcagtgactgtgacttggaactctggatccctgtc
cagcagtgtgcacaccttcccagctctcctgcagtctggactctacacta
tgagcagctcagtgactgtcccctccagcgcctggccaagtcagaccgtc
acctgcagcgttgctcacccggccagcaacaccacggtcgac

FIG. 33

17D2 - HC

gacgtccagatccagcagtctgggcctgagctggtgaagcctggagcttc 50
PCR primer

agtgaagatgtcctgcaaggcttctggttactcattcactgcctacaaca 100
CDR I

tgcactgggtgaagcagacccatggaaagagccttgagtggattggttat 150

attgatccttacagtggtgctactagctaccaccagaaattcaagggcaa 200
CDR II

ggccacattgactgttgacaaatcttccagcacagcctacatgcgcctca 250

acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg 300

gactgggacccttatgctatggactactggggtcaaggaacctcagtcac 350
CDR III

cgtctcctcagccaaaacaacaccccatcagtctatccactggcccctg 400
constant region

ggtgtggagatacaactggttcctccgtgactctgggatgcctggtcaag 450

ggctacttccctgagtcagtgactgtgacttggaactctggatccctgtc 500

cagcagtgtgcacaccttcccagctctcctgcagtctggactctacacta 550

tgagcagctcagtgactgtcccctccagcgcctggccaagtcagaccgtc 600

acctgcagcgttgctcacccggccagcaacaccacggtcgac 642
PCR primer

FIG. 34

17D2 - LC

agcgttgagatgtcacagtcgccagccatcctgtctgtgagtccaggaga
aagaatcagtttctcctgcagggccagtcagagcattggcacaagcatac
actggtatcagcaaagaacaaatggttctccaaggcttctcattaagtat
gcttctgcgtctatctctgggatcccttccaggtttagtggcagtggatc
agggacagattttactcttagcatcaacagtgtggagtctgaagatattg
cagattattactgtcaacaaagtaatagctggccgctcacgttcggtgct
gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat
cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt
gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt
gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga
cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg
acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca
tcaacttcacccattgtcaagagcttcaacaggaatgagtgt

FIG. 35

17D2 - LC

agcgttgagatgtcacagtcgccagccatcctgtctgtgagtccaggaga 50
PCR primer

aagaatcagtttctcctgcagggccagtcagagcattggcacaagcatac 100
CDR I

actggtatcagcaaagaacaaatggttctccaaggcttctcattaagtat 150

gcttctgcgtctatctctgggatcccttccaggtttagtggcagtggatc 200
CDR II

agggacagattttactcttagcatcaacagtgtggagtctgaagatattg 250

cagattattactgtcaacaaagtaatagctggccgctcacgttcggtgct 300
CDR III

gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat 350
constant region

cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt 400

gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt 450

gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga 500

cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg 550

acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca 600

tcaacttcacccattgtcaagagcttcaacaggaatgagtgt 642
PCR primer

FIG. 36

14D3 – HC

gacgtccagatgcagcagcctgggcctgagctggtgaagcctggagcttc
actaaagatgtcctgcaaggcttctggttactcattcactggctacaaca
tgcactgggtgaagcagagccatggaaagagccttgagtggattggatat
attgatccttacagtggtgctactagctacaaccagaaattcgagggcaa
ggccacattgactgtagacaaatcttccagcacagcctacatgcagctca
acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg
gactgggacccttatgctatggactactggggtcaaggaacctcagtcac
cgtctcctcagccaaaacaacagccccatcggtctatccactggcccctg
tgtgtggagatacaagtggctcctcggtgactctaggatgcctggtcaag
ggttatttccctgagccagtgaccttgacctggaactctggatccctgtc
cagtggtgtgcacaccttcccagctgtcctgcagtctgacctctacaccc
tcagcagctcagtgactgtaacctcgagcacctggcccagccagtccatc
acctgcaatgtggcccacccagccagcaacaccaaggtcgac

FIG. 37

14D3 - HC

gacgtccagatgcagcagcctgggcctgagctggtgaagcctggagcttc 50

PCR primer

actaaagatgtcctgcaaggcttctggttactcattcactggctacaaca 100

CDR I

tgcactgggtgaagcagagccatggaaagagccttgagtggattggatat 150

attgatccttacagtggtgctactagctacaaccagaaattcgagggcaa 200

CDR II

ggccacattgactgtagacaaatcttccagcacagcctacatgcagctca 250

acagcctgacatctgaggactctgcagtctattactgtgcaagaagatgg 300

CDR III

gactgggaccctatgctatggactactggggtcaaggaacctcagtcac 350

cgtctcctcagccaaaacaacagccccatcggtctatccactggcccctg 400

constant

tgtgtggagatacaagtggctcctcggtgactctaggatgcctggtcaag 450

ggttatttccctgagccagtgaccttgacctggaactctggatccctgtc 500

cagtggtgtgcacaccttcccagctgtcctgcagtctgacctctacaccc 550

tcagcagctcagtgactgtaacctcgagcacctggcccagccagtccatc 600

acctgcaatgtggcccacccagccagcaacaccaaggtcgac 642

PCR primer

FIG. 38

14D3 - LC

aacattctgatgacacagtctccagccatcttgtctgtgagtccaggaga
aagagtcagtttcgcctgcagggccagtcagagcattggcacaagcatac
actggtatcagcaaagaacaaatggttctccaaggcttctcataaagtat
gcttctgagtctatctctgggatcccttccaggtttagtggcagtggatc
agggacagattttactcttagcatcaacagtgtggagtctgaagatattg
cagattattactgtcaacaaactaataggtggccgctcacgttcggtgct
gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat
cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt
gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt
gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga
cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg
acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca
tcaacttcacccattgtcaagagcttcaacaggaatgagtgt

FIG. 39

14D3 - LC

aacattctgatgacacagtctccagccatcttgtctgtgagtccaggaga 50
PCR primer

aagagtcagtttcgcctgcagggccagtcagagcattggcacaagcatac 100
CDR I

agctggtatcagcaaagaacaaatggttctccaaggcttctcataaagtat 150

gcttctgagtctatctctgggatcccttccaggtttagtggcagtggatc 200
CDR II

agggacagattttactcttagcatcaacagtgtggagtctgaagatattg 250

cagattattactgtcaacaaactaataggtggccgctcacgttcggtgct 300
CDR III

gggaccaagctggagctgaaacgggctgatgctgcaccaactgtatccat 350
constant region

cttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt 400

gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagatt 450

gatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga 500

cagcaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg 550

acgagtatgaacgacataacagctatacctgtgaggccactcacaagaca 600

tcaacttcacccattgtcaagagcttcaacaggaatgagtgt 642
PCR primer

FIG. 40

3B3 - HC

DVQLQQPGAELVKPGASVKLSCTTSGVNIKDTYMHWMKQRPEQGLEWIGR
IDPANGNTKYDPKFRGKATITADTSSNTVYVQLRSLTSEDTAVYYCAYDG
YWGQGTLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVT
VTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPA
SSTKVD

FIG. 41

3B3 - HC

DVQLQQPGAELVKPGASVKLSCTTSGVNIKDTYMHWMKQRPEQGLEWIGR 50
PCR primer CDR I

IDPANGNTKYDPKFRGKATITADTSSNTVYVQLRSLTSEDTAVYYCAYDG 100
CDR II CDR III

YWGQGTLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVT 150
constant region

VTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPA200
PCR primer

206
SSTKVD

FIG. 42

3B3 - LC

NIVMTQTPASLAVSLGQRATISCRASESVDSYGNNFMHWYQQKPGQSPRL
LIYRASNLESGIPARFSGSGSRTDFTLTTNPVEADDVATYYCQQSHKDPL
TFGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV
KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA
THKTSTSPIVKSFKANEC

FIG. 43

3B3 - LC

NIVMTQTPASLAVSLGQRATISCRASESVDSYGNNFMHWYQQKPGQSPRL 50
PCR primer CDR I

LIYRASNLESGIPARFSGSGSRTDFTLTTNPVEADDVATYYCQQSHKDPL 100
CDR II CDR III

TFGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV 150
constant region

KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA 200

THKTSTSPIVKSFKANEC 218
PCR primer

FIG. 44

3C7 - HC

DVQLKHSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLDWIGL
INPYNGGTSYDQKFKGKATLTVDKSSSTAYMELLSLTSEDSAVYYCARDG
LMDYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA
HPASKTKVD

FIG. 45

3C7 - HC

DVQLKHSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLDWIGL 50
PCR primer
CDR I

INPYNGGTSYDQKFKGKATLTVDKSSSTAYMELLSLTSEDSAVYYCARDG 100
CDR II

LMDYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE 150
CDR III
constant region

PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA 200

HPASKTKVD 209
PCR primer

FIG. 46

3C7 - LC

DIVMTQTPASLAVSLGQRATIFCRASQSVDYNGISYMHWFQQKPGQPPKL
LIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSFEDPH
TFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV
KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA
THKTSTSPIVKSFNRNEC

FIG. 47

3C7 - LC

DIVMTQTPASLAVSLGQRATIFCRASQSVDYNGISYMHWFQQKPGQPPKL 50
PCR primer CDR I

LIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSFEDPH 100
CDR II CDR III

IFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV 150
constant region

KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA 200

THKTSTSPIVKSFNRNEC 218
PCR primer

FIG. 48

2B4 - HC

DVQLQQSGTVLARPGASVRMSCKASGYSFTRYWIHWLKQRPGQGLEWIGA
IFPGNRDTSYNQRFKGKAEVTAVTSASTAYLDLSSLTNEDSAVYYCTRWP
YYGSIYVNFDYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCL
VKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSE
TVTCNVAHPASSTKVD

FIG. 49

2B4 - HC

DVQLQQSGTVLARPGASVRMSCKASGYSFTRYWIHWLKQRPGQGLEWIGA 50
PCR primer CDR I

IFPGNRDTSYNQRFKGKAEVTAVTSASTAYLDLSSLTNEDSAVYYCTRWP 100
CDR II

YYGSIYVNFDYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCL 150
CDR III constant region

VKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSE 200

TVTCNVAHPASSTKVD 216
PCR primer

FIG. 50

2B4 - LC

DIVMTQSPLSLPVSLGDQASISCRTSQNLVHRNGNTYLHWYLQKPGQSPK
LLIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQGTHVP
PTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDIN
VKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCE
ATHKTSTSPIVKSFNRNEC

FIG. 51

2B4 - LC

DIVMTQSPLSLPVSLGDQASISCRTSQNLVHRNGNTYLHWYLQKPGQSPK 50
PCR primer CDR I

LLIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQGTHVP 100
CDR II CDR III

PTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDIN 150
constant region

VKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCE 200

ATHKTSTSPIVKSFNRNEC 219
PCR primer

FIG. 52

3B3 - HC

gacgtccagctccagcagcctggagcagagcttgtgaagccaggggcctc
agtcaagttgtcctgcaccacttctggcgtcaacattaaagacacctata
tgcactggatgaagcagaggcctgaacagggcctggagtggattggaagg
attgatcctgcgaatggtaatactaaatatgacccgaaattccggggcaa
ggccactataacagcagacacatcctccaacacggtctacgtgcaactca
gaagcctgacatctgaggacactgccgtctattactgtgcctatgatggt
tactggggccaagggactctggtcactgtctctgcagccaaaacgacacc
cccatctgtctatccactggcccctggatctgctgcccaaactaactcca
tggtgaccctgggatgcctggtcaagggctatttccctgagccagtgaca
gtgacctggaactctggatccctgtccagcggtgtgcacaccttcccagc
tgtcctgcagtctgacctctacactctgagcagctcagtgactgtcccct
ccagcacctggcccagcgagaccgtcacctgcaacgttgcccacccggcc
agcagcaccaaggtcgac

FIG. 53

3B3 - HC

gacgtccagctccagcagcctggagcagagcttgtgaagccaggggcctc 50
PCR primer

agtcaagttgtcctgcaccacttctggcgtcaacattaaagacacctata 100
CDR I

tgcactggatgaagcagaggcctgaacagggcctggagtggattggaagg 150

attgatcctgcgaatggtaatactaaaatatgacccgaaattccgggggcaa 200
CDR II

ggccactataacagcagacacatcctccaacacggtctacgtgcaactca 250

gaagcctgacatctgaggacactgccgtctattactgtgcctatgatggt 300
CDR III

tactggggccaagggactctggtcactgtctctgcagccaaaacgacacc 350
constant region

cccatctgtctatccactggcccctggatctgctgcccaaactaactcca 400

tggtgaccctgggatgcctggtcaagggctatttccctgagccagtgaca 450

gtgacctggaactctggatccctgtccagcggtgtgcacaccttcccagc 500

tgtcctgcagtctgacctctacactctgagcagctcagtgactgtcccct 550

ccagcacctggcccagcgagaccgtcacctgcaacgttgcccacccggcc 600
PCR primer

agcagcaccaaggtcgac 618

FIG. 54

3B3 - LC

aacattgtgatgacccaaactccagcctctttggctgtgtctctagggca
gagggccaccatatcctgcagagccagtgaaagtgttgatagttatggca
ataattttatgcactggtaccagcagaaaccaggacagtcacccagactc
ctcatctatcgtgcatccaacctagaatctgggatccctgccaggttcag
tggcagtgggtctaggacagacttcaccctcaccactaatcctgtggagg
ctgatgatgttgcaacctattactgtcagcaaagtcataaggatccgctc
acgttcggtgctgggaccaagctggagctgaaacgggctgatgctgcacc
aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg
cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc
aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg
gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca
cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc
actcacaagacatcaacttcacccattgtcaagagcttcaaggaacatga
gtgt

FIG. 55

3B3 - LC

aacattgtgatgacccaaactccagcctctttggctgtgtctctagggca 50

PCR primer

gagggccaccatatcctgcagagccagtgaaagtgttgatagttatggca 100

CDR I

ataatttatgcactggtaccagcagaaaccaggacagtcacccagactc 150

ctcatctatcgtgcatccaacctagaatctgggatccctgccaggttcag 200

CDR II

tggcagtgggtctaggacagacttcaccctcaccactaatcctgtggagg 250

ctgatgatgttgcaacctattactgtcagcaaagtcataaggatccgctc 300

CDR III

acgttcggtgctgggaccaagctggagctgaaacgggctgatgctgcacc 350

constant region

aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg 400

cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc 450

aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg 500

gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca 550

cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc 600

actcacaagacatcaacttcacccattgtcaagagcttcaacaggaatga 650

PCR primer

gtgt 652

FIG. 56

3C7 - HC

gacgtccagctgaagcatcaggacctgagctggtgaagcctggagcttca
atgaagatatcctgcaaggcttctggttactcattcactggctacaccat
gaactgggtgaagcagagccatggaaagaaccttgagtggattggactta
ttaatccttacaatggtggtactagctacgaccagaagttcaagggcaag
gccacattaactgtagacaagtcatccagcacagcctacatggagctcct
cagtctgacatctgaggactctgcagtctattactgtgcaagagatggcc
tgatggactactggggtcaaggaacctcagtcaccgtctcctcagccaaa
acgacacccccatctgtctatccactggcccctggatctgctgcccaaac
taactccatggtgaccctgggatgcctggtcaagggctatttccctgagc
cagtgacagtgacctggaactctggatccctgtccagcggtgtgcacacc
ttcccagctgtcctgcagtctgacctctacactctgagcagctcagtgac
tgtcccctccagcacctggcccagcgagaccgtcacctgcaacgttgccc
acccggccagcaagaccaaggtcgac

FIG. 57

3C7 - HC

gacgtccagctgaagcatcaggacctgagctggtgaagcctggagcttca 50

PCR primer

atgaagatatcctgcaaggcttctggttactcattcactggctacaccat 100

CDR I

gaactgggtgaagcagagccatggaaagaaccttgagtggattggacttta 150

CDR II

ttaatccttacaatggtggtactagctacgaccagaagttcaagggcaag 200

gccacattaactgtagacaagtcatccagcacagcctacatggagctcct 250

cagtctgacatctgaggactctgcagtctattactgtgcaagagatggcc 300

CDR III

tgatggactactggggtcaaggaacctcagtcaccgtctcctcagccaaa 350

constant region

acgacaccccatctgtctatccactggccctggatctgctgcccaaac 400

taactccatggtgaccctgggatgcctggtcaagggctatttccctgagc 450

cagtgacagtgacctggaactctggatccctgtccagcggtgtgcacacc 500

ttcccagctgtcctgcagtctgacctctacactctgagcagctcagtgac 550

tgtcccctccagcacctggcccagcgagaccgtcacctgcaacgttgccc 600

acccggccagcaagaccaaggtcgac 626

PCR primer

FIG. 58

3C7 - LC

gatattgtgatgacccaaactccagcttctttggctgtgtctctaggaca
gagagccactatcttctgcagagccagccagagtgtcgattataatggaa
ttagttatatgcactggttccaacagaaaccaggacagccacccaaactc
ctcatctatgctgcatccaacctagaatctgggatccctgccaggttcag
tggcagtgggtctgggacagacttcaccctcaacatccatcctgtggagg
aggaagatgctgcaacctattactgtcagcaaagttttgaggatccgcac
acgttcggaggggggaccaagctggaaataaaacgggctgatgctgcacc
aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg
cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc
aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg
gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca
cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc
actcacaagacatcaacttcacccattgtcaagagcttcaacaggaatga
gtgt

FIG. 59

3C7 - LC

gatattgtgatgacccaaactccagcttctttggctgtgtctctaggaca 50
PCR primer

gagagccactatcttctgcagagccagccagagtgtcgattataatggaa 100
CDR I

ttagttatatgcactggttccaacagaaaccaggacagccacccaaactc 150

ctcatctatgctgcatccaacctagaatctgggatccctgccaggttcag 200
CDR II

tggcagtgggtctgggacagacttcaccctcaacatccatcctgtggagg 250

aggaagatgctgcaacctattactgtcagcaaagttttgaggatccgcac 300
CDR III

acgttcggaggggggaccaagctggaaataaaacgggctgatgctgcacc 350
constant region

aactgtatccatcttcccaccatccagtgagcagttaacatctggaggtg 400

cctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtc 450

aagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttg 500

gactgatcaggacagcaaagacagcacctacagcatgagcagcaccctca 550

cgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggcc 600

actcacaagacatcaacttcacccattgtcaagagcttcaacaggaatga 650
PCR primer

gtgt 654

FIG. 60

2B4 - HC

gacgtccagctgcagcagtctgggactgtgctggcaaggcctggggcttc
cgtgaggatgtcctgcaaggcttctggctacagctttaccaggtactgga
tacactggttaaaacagaggcctggacagggtctagaatggattggtgct
atttttcctggaaatcgtgataccagttacaaccagaggttcaagggcaa
ggccgaagtgactgcagtcacatccgccagcactgcctacttggacctca
gtagcctgacaaatgaggactctgcggtctattactgtacaagatggcct
tactatggttccatctacgttaactttgactactggggccaaggcaccac
tctcacagtctcctcagccaaaacgacacccccatctgtctatccactgg
cccctggatctgctgcccaaactaactccatggtgaccctgggatgcctg
gtcaagggctatttccctgagccagtgacagtgacctggaactctggatc
cctgtccagcggtgtgcacaccttcccagctgtcctgcagtctgacctct
acactctgagcagctcagtgactgtcccctccagcacctggcccagcgag
accgtcacctgcaacgttgcccacccagccagcagcaccaaggtcgac

FIG. 61

2B4 - HC

gacgtccagctgcagcagtctgggactgtgctggcaaggcctggggcttc 50
PCR primer

cgtgaggatgtcctgcaaggcttctggctacagctttaccaggtactgga 100
CDR I

tacactggttaaaacagaggcctggacagggtctagaatggattggtgct 150

attttcctggaaatcgtgataccagttacaaccagaggttcaagggcaa 200
CDR II

ggccgaagtgactgcagtcacatccgccagcactgcctacttggacctca 250

gtagcctgacaaatgaggactctgcggtctattactgtacaagatggcct 300

tactatggttccatctacgttaactttgactactggggccaaggcaccac 350
CDR III

tctcacagtctcctcagccaaaacgacaccccatctgtctatccactgg 400
constant region

cccctggatctgctgcccaaactaactccatggtgaccctgggatgcctg 450

gtcaagggctatttccctgagccagtgacagtgacctggaactctggatc 500

cctgtccagcggtgtgcacaccttcccagctgtcctgcagtctgacctct 550

acactctgagcagctcagtgactgtcccctccagcacctggcccagcgag 600

accgtcacctgcaacgttgcccacccagccagcagcaccaaggtcgac 648
PCR primer

FIG. 62

2B4 - LC

gatattgtgatgacccagtctcctctctccctgcctgtcagtcttggaga
tcaagcctccatctcttgcagaactagtcagaaccttgtacacaggaatg
gaaacacctatttacattggtacctgcagaagccaggccagtctccaaag
ctcctgatttacaaaatttccaaccgattttctggggtcccagacaggtt
cagtggcagtggatcagggacagatttcacactcaagatcagcagagtgg
aggctgaggatctgggagtttatttctgctctcaaggtacacatgttcct
ccgacgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgc
accaactgtatccatcttcccaccatccagtgagcagttaacatctggag
gtgcctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaat
gtcaagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacag
ttggactgatcaggacagcaaagacagcacctacagcatgagcagcaccc
tcacgttgaccaaggacgagtatgaacgacataacagctatacctgtgag
gccactcacaagacatcaacttcacccattgtcaagagcttcaacaggaa
tgagtgt

FIG. 63

2B4 - LC

gatattgtgatgacccagtctcctctctccctgcctgtcagtcttggaga 50
PCR primer

tcaagcctccatctcttgcagaactagtcagaaccttgtacacaggaatg 100
CDR I

gaaacacctatttacattggtacctgcagaagccaggccagtctccaaag 150

ctcctgatttacaaaaatttccaaccgatttctggggtcccagacaggtt 200
CDR II

cagtggcagtggatcagggacagatttcacactcaagatcagcagagtgg 250

aggctgaggatctgggagtttatttctgctctcaaggtacacatgttcct 300
CDR III

ccgacgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgc 350
constant region

accaactgtatccatcttcccaccatccagtgagcagttaacatctggag 400

gtgcctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaat 450

gtcaagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacag 500

ttggactgatcaggacagcaaagacagcacctacagcatgagcagcaccc 550

tcacgttgaccaaggacgagtatgaacgacataacagctatacctgtgag 600

gccactcacaagacatcaacttcacccattgtcaagagcttcaacaggaa 650
PCR primer

tgagtgt 657

FIG. 64